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Utilization of phosphorus in poultry as influenced by dietary calcium and phosphorus source

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UTILIZATION OF PHOSPHORUS IN POULTRY AS INFLUENCED BY DIETARY CALCIUM AND PHOSPHORUS SOURCE

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Utilization of phosphorus in poultry as influenced by dietary calcium and phosphorus source

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GENERAL INTRODUCTION

The element phosphorus is a vital constituent of all living cells in the avian body. Phosphorus functions as a constituent of bone and is a component of organic compounds involved in almost every aspect of energy, carbohydrate, amino acid, and fat metabolism, nervous tissue metabolism, blood chemistry and lipid transport. Phosphates are part of nucleic acids (DNA and RNA), components of coenzymes, and a part of high energy compounds such as ATP and creatine phosphate.

Phosphorus is an essential mineral in the poultry diet. All birds have a requirement for phosphorus and this requirement can be affected by numerous factors such as environmental conditions, genetic strain and other nutrients in the diet. The National Research Council (1984) recommended dietary phosphorus level for laying hens is .32% available phosphorus per kilogram of diet. The term "available" means that portion of the total phosphorus in the bird's diet which is relatively available for absorption and utilization. Unavailable phosphorus is usually defined as that phosphorus which is associated with phytate (organic form of phosphorus found in plants) in the diet. Phytate phosphorus is regarded as being unavailable for absorption in monogastric animals because these animals do not have the enzyme phytase in the gastrointestinal tract. However, there is disagreement among researchers concerning the ability of poultry to utilize phytate phosphorus. Some researchers have reported that as much as 40% phytate P consumed is utilized by poultry.

The phosphorus requirement of poultry will vary with the age of the

bird. Young growing poultry have a higher phosphorus requirement than adults because of the phosphorus needs for skeletal growth. The laying hen needs phosphorus, not only for maintenance of skeleton and soft tissues, but also for egg production. Each egg produced contains approximately 80 to 120 mg phosphorus. Thus, the laying hen's phosphorus requirement can vary according to her rate of daily egg production. The concept of phase feeding phosphorus is based on changes in the phosphorus requirement of laying hens for egg production. As hens age, egg production decreases and the need for dietary phosphorus also decreases. Phase feeding is a management practice which decreases the level of phosphorus supplemented to laying hen diets according to the age and rate of egg production of the laying hen. Phase feeding programs usually offer economic incentive by reducing the costs of phosphorus supplementation in hen diets.

Actual phosphorus requirements of poultry as influenced by the aforementioned factors are difficult to measure accurately. Many researchers have utilized balance techniques to determine phosphorus requirements. However, a thorough assessment of phosphorus requirement by balance techniques at various stages of a hen's production period has not been reported in the literature.

The objectives of this dissertation were to conduct a series of experiments to: 1. assess the utilization of total phosphorus and phytate phosphorus in the laying hen as influenced by dietary calcium, phase feeding phosphorus programs, strain of hen and age of hen; 2. test the effects of dietary calcium on ^{33}P distribution and phytate phosphorus

utilization in young broiler chicks and, 3. determine the effects of dietary calcium on phosphorus absorption and excretion in the laying hen.

Explanation of Dissertation Format

The three sections of this dissertation represent three complete manuscripts which have already or will be submitted for publication in scientific journals under the authorship of Sheila E. Scheideler and Jerry L. Sell.

LITERATURE REVIEW

Phosphorus is the element P with an atomic weight of 30.975. Phosphorus does not occur free in nature, but is found in the form of phosphates in inorganic and organic sources. The laying hen has a minimum concentration of phosphate in her organs to maintain normal cellular function. Phosphorus or phosphates are components of organic compounds involved in almost every metabolic pathway. Phosphate functions in acid-base balance, metabolic regulation, as well as a component of nucleic acids, proteins, lipids, and bone.

Phosphorus Requirement of the Laying Hen

The dietary P requirement of an individual laying hen represents the amount of P the hen needs to consume each day to replace losses via feces, urine and the egg (Garlich, 1979). Diets must be formulated to contain a minimum quantity of biologically available P to maintain a stable P balance in the laying hen. The National Research Council's (NRC) (1984) recommended requirement for P of the laying hen is .32% dietary available phosphorus (AP) based on a feed intake of 110 g/hen daily or 350 mg AP intake/hen daily. AP includes that P in a feed supplied by inorganic supplements, such as dicalcium phosphate, or from animal sources, such as meat and bone meal. P from these sources is considered to be 100% available, while P from feed ingredients of plant origin is assumed to only be 30% available (Scott et al., 1976). Total phosphorus (TP) is defined as the total mineral P in a ration, regardless of its availability to the laying hen (Scott et al., 1976). The differ-

ence between AP and TP is the amount of phytate P in the ration, which is assumed to be unavailable to the hen. Approximately two-thirds of the P in plant sources is in the form of phytate P.

This literature review will refer to TP or AP according to the author's use. Edwards (1978) did an extensive review of the literature and observed that the minimum requirement for TP in most reports was the lowest level of TP tested, which made estimation of the requirement difficult. Edwards concluded from his review that the minimum AP requirement for laying hens was in the range of 0.18 to 0.25% AP, which is equivalent to 0.36 to 0.43% TP in corn-soybean meal diets.

An early study by Singsen et al. (1962) reported the highest egg production (79.5%) in hens fed 0.50% TP, but this rate was not significantly different from those of hens fed .40, .50, or .60% TP. Singsen calculated the point of maximum response at 0.53% TP (0.48% AP). Egg production was significantly depressed in hens fed 0.70% TP.

Summers et al. (1976) fed 0.15% to 0.55% AP to the laying hen and reported no significant improvement in hen day egg production above 0.25% AP, despite an increase in egg weight and body weight at 0.55% AP.

Owings et al. (1977) conducted two experiments in which 22 and 47-week-old hens were fed 0.19, 0.28 or 0.37% AP for 140 days. In the first experiment, a high rate of egg production (>78%) was reported at all levels of dietary P; however, the mortality rate was slightly higher for hens fed 0.19% AP. In the second experiment utilizing 47-week-old laying hens, a basal ration contained 0.10% AP (0.30% TP all from plant sources), and three additional diets were obtained by adding 0.09, 0.18

or 0.27% AP to the basal. Egg production by hens fed the 0.10% AP basal diet decreased within 4 weeks while hens fed 0.19% or more AP maintained a high rate of egg production for 56 days. Hens fed 0.10% AP also lost considerable body weight and had a high rate of mortality.

Phosphorus requirement was estimated by Hurwitz and Griminger (1962) by use of a balance technique. They fed a purified diet with graded amounts of P from sodium and potassium hydrogen phosphates for 9 days to 13 to 15 month-old hens laying at a rate of 70 to 80% and estimated a P requirement of 240 to 360 mg P/hen/day. This range corresponds well with the current NRC requirement of 350 mg/day intake of AP.

Recently, Edwards (1986) tested the adequacy of five different levels of TP (0.3, 0.4, 0.5, 0.6, and 0.7) for laying hens. The corn-soybean meal ration containing no supplemental inorganic P (.30% TP) was unable to support maximum egg production in young pullets. An additional .20% inorganic P (.50% TP) was adequate to maintain maximum egg production for over 1 year and .40% TP resulted in near maximum egg production. Edwards concluded from this trial that the hen's requirement for non-phytate P was .33% or 330 mg/day for maximum egg production. Furthermore, the .70% TP diet actually decreased egg production, resulting in a quadratic effect of dietary P on egg production.

Factors Affecting P Utilization in the Laying Hen

A number of factors affect the hens ability to utilize dietary P. These factors include: interactions with other nutrients, genetic strain, environmental conditions and management.

Interactions with other nutrients

Calcium A proper ratio of calcium (Ca) to P is vital, especially in young poultry. In growing birds, a ratio of about 1:1.2 is considered to be ideal; however, ratios of 1:1 to 1:5 are well tolerated (NRC, 1977). A higher Ca level is necessary in laying hen diets to support egg production. In this instance, the ratio increases to 1:4 or more. The P:Ca ratio seems to be more critical in growing versus adult birds. Various studies have demonstrated a wide range of dietary Ca and P levels that adequately support the productivity of laying hens. However, various researchers have reported conflicting data on the effect of Ca on P utilization and the interaction effects of dietary Ca and P on the production traits of laying hens.

Hurwitz and Bar (1965) found that the percent P absorption by hens was depressed as dietary Ca increased from 1.9% to 3.56%. These authors also observed a considerable endogenous P excretion by hens fed the low Ca diet, indicating mobilization of medullary bone. Ademosun and Kalango (1973) fed pullets 2.0, 2.75, 3.5 and 4.25% Ca in a factorial combination with .4 and .6% TP. They found egg production to increase as Ca increased from 2.0 to 3.5%, and to be greater for pullets fed .60% versus .40% TP, only the P main effect was statistically significant.

Dietary Ca can also affect other production characteristics such as plasma levels of P and egg specific gravity which are dependent on P utilization. Reichmann and Connor (1977) reported an interaction between dietary Ca and P on plasma inorganic P. With 2.4% Ca and .45% P, plasma P was atypically low at 36.2 mg P/l. In contrast, hens fed .60% P and

2.5% Ca, had a plasma P level of 45.9 mg/l. Miles and Harms (1982) fed hens two levels of Ca (3.25 and 4.65%) and two levels of TP (.42 and 1.40%) and reported the highest specific gravity in eggs produced by hens consuming 4.65% Ca in the presence of .42% TP. Increasing dietary TP from .42 to 1.40% caused a significant decrease in egg specific gravity indicating an adverse effect on shell quality.

Outerhout (1980) fed dietary Ca ranging from 2.75 to 4.75% and .35 or .45% AP. No significant Ca X P interaction effects were observed on egg production, egg weight, eggshell weight, or egg specific gravity. These observations contrast with those of Summers et al. (1976), who reported significant interaction effects between level of dietary Ca and P on feed consumption and egg weight. A low Ca level (1.50%) in the presence of high P (.57%) reduced feed consumption as compared with the 1.50% Ca and .42% TP group. Low Ca resulted in reduced egg weight only in the presence of low P. Summers et al. (1976) also reported an interaction between dietary Ca and P with respect to P retention. High levels of Ca (2.96%) and P (.57%) resulted in low P retention (3.7%) while high Ca (2.96%), low P (.42%) or low Ca (1.50%) and high P (.57%) gave P retention values of 11.0 and 10.7%, respectively. Diets with low Ca (1.50%) and low P (.42%) resulted in negative P retention.

Keshavarz (1986) fed three levels of Ca (3.5, 4.5, and 5.5%) and three levels of AP (.24, .44, and .64%) to 56 week old hens. Absolute P retention decreased from .23 to .19 and .13 g/day as dietary Ca increased from 3.5 to 4.5 and 5.5%, respectively. Dietary Ca had no effect on production variables, plasma P, egg shell quality, or tibia ash in this

experiment. A significant interaction effect of Ca X P on % and absolute P retention existed, in which hens fed 3.5 and 5.5% Ca had decreased % P retention as dietary P increased from .24 to .64%, whereas hens fed 4.5% Ca had greater P retention when fed .44 and .64% P versus .24% P.

Vitamin D Vitamin D functions as a hormone in Ca and P metabolism. The physiologic effect of Vitamin D is to facilitate bone calcification by increasing the absorption of dietary Ca. The mechanism of Ca absorption and the role of vitamin D in this mechanism is well established. Vitamin D stimulates synthesis of a calcium binding protein which facilitates the uptake of Ca by intestinal mucosa. When insufficient dietary Ca is available, vitamin D will have a direct effect on bone and cause bone mobilization, thereby increasing endogenous Ca and P loss.

Several researchers have reported a vitamin D effect on intestinal P absorption in the chick. Wasserman and Taylor (1973) and Wasserman (1981) have demonstrated active transport of phosphate at the brush border of the intestinal cell under the control of vitamin D. Fuchs and Peterlik (1980) reported that vitamin D stimulated intestinal cellular uptake of phosphate twofold in rats at the site of the brush border membrane. Lee et al. (1986), found active P absorption in young vitamin D deficient rats, while active intestinal P secretion was found in vitamin D deficient adult rats, indicating regulation of intestinal P transport by age-dependent, but vitamin D independent mechanisms. However, the authors also found increased jejunal P absorption in P deficient rats only when vitamin D was repleted.

Energy Metabolizable energy level of the diet can cause changes in feed intake which can, in turn, influence the intake of P by the laying hen (Edwards, 1978). Therefore, appropriate adjustment in dietary P concentration may be necessary to compensate for changes in feed intake caused by dietary energy.

Strain of hen

Strain differences in P utilization and P requirement have been reported by several researchers. Edwards (1982) compared Single Comb White Leghorn (SCWL) cockerel chickens with broiler chickens in their ability to utilize suboptimal levels of dietary P. The SCWL chickens had higher Ca, P and phytin P retentions than the broiler strain. The requirement for non-phytate P for maximum bone ash was .38 to .41% AP for SCWL chickens versus .43 to .45% AP for the broiler chickens. Edwards (1982) concluded that the major difference between the two breeds was their ability to utilize phytate P. The SCWL chickens retained much larger quantities of phytate P than the broiler chicken.

Edwards and Suso (1981) conducted a similar experiment estimating the P requirement of six strains of caged laying hens. In this study, no interaction effects on production characteristics were seen between strain and dietary P. The P requirement of all six strains was estimated at .45% TP. Strain differences were seen only in hen weight and egg production rate.

Rodriguez et al. (1984) conducted an experiment measuring the influence of phase feeding AP on production characteristics of three strains of SCWL laying hens. No strain X dietary P interaction effects

on production traits were detected. However, significant strain differences were noted in feed efficiency, egg weight, carcass ash and carcass P.

Environmental conditions

Heat stress seems to be one of the most influential environmental factors affecting P utilization in poultry. Garlich et al. (1977) reported increased mortality among a flock of laying hens subjected to heat stress and fed experimental low P diets. In subsequent trials with chicks, Garlich et al. (1977) found that the mortality rate of chicks subjected to heat stress was related to their ability to maintain an adequate plasma inorganic P concentration. This concept was supported by the research findings of Charles et al. (1978) who reported increased mortality among laying hens having low P intakes during periods of extreme heat. This effect was caused by decreased feed intake at high temperatures. Charles and Duke (1982) reported a similar experiment in which high temperature (30°C) increased the laying hen's P requirement, as a % of diet, above that required in a cool (10°C) environment. The increase in P requirement during heat stress is likely due to reduced feed intake and insufficient P consumption during high temperatures.

Management practices

Phase feeding is a management practice used in poultry nutrition to minimize feed costs while maintaining an adequate nutrient intake. Harms (1979 and 1986) recommended the use of phase feeding regimes to laying hens. Harms (1979) suggested daily intakes of 650, 550, and 450 mg

TP/hen/day for the age periods of 20 to 36, 37 to 52 and 53 weeks onward, respectively. The highest levels of TP were recommended for the first 16 weeks of lay for the prevention of cage layer fatigue. A low level of TP was recommended after 53 weeks of age to improve egg shell quality.

Mikaelian and Sell (1981) fed hens diets containing .46, .36, and .26% AP or .36, .26, and .16% AP for ages 27 to 39, 39 to 51, and 51 to 71 weeks, respectively. No significant effects of dietary AP were observed on egg production, feed efficiency, egg weight, egg shell thickness, body weight or mortality.

Said and Sullivan (1982) conducted a study in which hens were phase-fed .60, .55, .50, and .45% TP or .55, .50, .45, and .40% TP for intervals of 20 to 32, 33 to 44, 45 to 56, and 57 to 68 weeks of age, respectively. The phase feeding TP regimens enhanced egg specific gravity at 44, 56 and 68 weeks of age, as well as bone ash at 68 weeks of age above that observed in hens continuously fed .40 to .69% TP. Feed consumption was increased and feed efficiency decreased with phase feeding TP regimens versus continuous feeding of TP. This contradicts data reported by Rodriguez et al. (1984) in which hens phase-fed AP levels of .35, .25, and .15% during age intervals of 22 to 34, 34 to 50 and 50 to 70 weeks, respectively, had a superior feed efficiency as compared with hens fed .15, .30, or .45% AP continuously from 22 to 70 weeks of age. Rodriguez et al. (1984) also found lighter body weights, less carcass ash and carcass P in phase-fed hens at the end of the experiment.

Daghir and Farran (1983) compared phase-feeding programs and contin-

uous AP feeding of hens in two experiments, one in cages and one in floor pens. Birds raised on the floor required a minimum of .25% AP for best egg production, feed conversion, body weight and egg shell thickness. The phase feeding program of .45, .35 and .25% AP gave the best response in these criteria for hens raised on the floor. Hens raised in cages at a temperature of 31°C. required a minimum of .35% AP for optimum egg production and feed conversion.

Another type of laying hen feeding program which could influence P utilization is the practice of feeding hens a Ca supplement separate from a low Ca basal ration. This program allows the hen to selectively consume Ca according to her needs at egg shell calcification times. Cabrera et al. (1982) conducted an experiment comparing the effects of a normal Ca diet (3.7% Ca) versus a totally separate Ca feeding on corrected metabolizable energy, N, Ca and P retention. Phosphorus and N retention were not significantly affected by separate Ca feeding. Corrected metabolizable energy was increased and Ca retention was negative on non-egg laying days in hens fed the separate Ca supplement.

Phosphorus Retention and Balance

Definition

Hurwitz and Griminger (1962) defined retention as the difference between the amount of a substance consumed and that excreted in urine and feces. Percent retention refers to the retention as a percentage of the total intake of this substance. Balance refers to retention minus loss of the substance in eggs produced. Hurwitz and Griminger (1962)

conducted a balance study in laying hens to determine their P requirement. They found that P excretion decreased linearly as dietary P was lowered. Percentage P retention was not closely related to P intake although the highest % retention was observed at the lowest level of P fed. Positive P balance was maintained by hens receiving .43 and .32% P, but lower levels were associated with a negative P balance. Hurwitz and Griminger (1962) concluded that the P requirements for egg formation and maintenance were not entirely independent nor were they additive.

Summers et al. (1976) conducted a series of experiments with laying hens in which they fed varying levels of dietary P and measured P retention. In two of the experiments, % P retention was less than 10% and, in many instances, retention was negative. Summers concluded that, irrespective of the level of dietary P, the laying hen only absorbs or retains very small quantities of P.

Keshavarz (1986) measured P retention in laying hens fed varying levels of dietary Ca and P. Phosphorus retention ranged from .13 to .23 g/day and % P retention ranged from 20.2 to 31.3%. The absolute retention of P decreased with increasing levels of dietary Ca. Increasing dietary P caused a decrease in P retention. Significant interactions between dietary Ca and P were observed on % and absolute retention. These interactions were discussed in a previous section of this literature review.

Phosphorus absorption

Hurwitz and Bar (1965) measured the absorption of P along the gastrointestinal tract of the laying hen, as influenced by dietary Ca and

egg shell formation. The main site for P absorption was the anterior intestine (jejunum), while, in the duodenum, an excess of P was present, indicating considerable endogenous P excretion in this segment. There was an increase in P absorption in the lower portion of the intestine during egg shell calcification; however, high dietary Ca decreased P absorption in the lower intestine, irrespective of shell calcification.

In a 1971 study, Hurwitz and Bar conducted trials with chicks and laying hens investigating the solubility and interrelationships of intestinal Ca and P. A general pattern of association between Ca and P existed in the intestinal contents. The bulk of Ca and P absorption occurred in the proximal small intestine; while in the duodenum (pH=6.5), a precipitation of Ca and P occurred, which caused decreased levels of ultrafilterable Ca and P. Hurwitz and Bar (1971) concluded that the quantitative relationships between Ca and P resulted from chemical association in the intestinal lumen rather than from interaction at the absorption site.

Intestinal P transport across the brush border of the intestinal cell is accomplished by a "phosphate pump" on the mucosal surface of the intestinal epithelium, (Fuchs and Peterlik, 1980). The phosphate pump is dependent on a sodium flux and is regulated by the presence of vitamin D (Fuchs and Peterlik, 1980 and Murer et al., 1981).

Wasserman and Taylor (1973) measured intestinal absorption of P in chicks and observed rapid ^{32}P translocation across all segments of the small intestine (duodenum, jejunum, ileum). Vitamin D positively affected absorption in each segment. On a surface area basis, the

duodenum was more efficient in absorbing ^{32}P than either the jejunum or the ileum. The authors also reported that with increasing concentrations of stable P, the rate of 3^2P absorption tended to decrease.

Fox and Care (1978) fed chicks diets low in Ca (0.1%) and(or) low in P (.25%) and found enhanced P absorption in the duodenum and ileum. The authors remarked that the increased P absorption in response to low Ca or low P diets may have been caused by stimulation of 1,25 (OH) D_3 activity due to low plasma Ca.

Phosphorus excretion

Phosphorus excretion is difficult to measure in poultry because of the combined excretion of urine and fecal matter through the cloaca. To measure urinary excretion of a nutrient, a colostomy can be performed to isolate the colon from the cloaca. A colostomy enables one to separately collect urine and feces. Hurwitz and Griminger (1961a) colostomized laying hens and measured P excretion. Phosphorus excretion in the urine of these hens was 51% of the ingested P and 62% of the total excreted P. When Ca balance in these hens was negative, indicating use of bone Ca for egg formation, the extra P mobilized from bone led to increased urinary P excretion and consequently a low P retention. Brown and McCracken (1965) utilized an isotope dilution technique to determine endogenous fecal P loss in laying hens. The authors reported that 33 mg fecal P/day was of endogenous origin, accounting for 9% of the total fecal P output. The endogenous P loss varied with the hen's requirement for bone development and egg formation.

Phosphorus excretion in colostomized hens was also measured by

Taylor and Kirkley (1967). They reported an increase in net absorption of P on egg laying days. Concurrently, because of bone mobilization on egg laying days excretion of P in urine also increased. Taylor and Kirkley (1967) reported that a high Ca diet decreased both bone mobilization and urinary P excretion. The authors noted that it may be possible for the cecum and rectum to absorb ions from the urine following a retrograde movement of matter from the cloaca into these regions.

Parathyroid hormone regulates P secretion and reabsorption mechanisms in the kidney (Martindale, 1973; and Klinefelter et al., 1984). Martindale (1973) studied P excretion in the laying hen and found that a parathyroid extract increased P excretion and appeared to inhibit both reabsorption and secretion. Klinefelter et al. (1984) reported that parathyroid hormone caused the kidneys of domestic fowl to secrete P into the urine and that the P pools in the kidney may serve as a source for the P secreted in response to parathyroid hormone. It is also likely that parathyroid hormone may be affecting P excretion via its role in Ca reabsorption and bone mobilization. When serum Ca is low, parathyroid hormone production is stimulated and acts on the bone to increase Ca mobilization. When the bone Ca is mobilized, P is mobilized too, as part of the hydroxyapatite crystal. The excess P is then excreted via urine.

Bioavailability of Phytate and Non-phytate Sources of Phosphorus

Two forms of P commonly found in poultry diets are phytate (organic) and non-phytate (inorganic) sources of P. Native inorganic phosphates derived from soils are relatively unavailable. Heat treatment will convert them to more highly available forms (tricalcium phosphate,

B-tricalcium phosphate) as compared to highly available P in animal by-products (Scott et al. 1976). Table 1 lists the comparative biological value of inorganic phosphates on the basis of bone calcification in chicks, with B-tricalcium phosphate assigned a value of 100 (Scott et al. 1976).

Phytate P (organic P) is the principal storage form of P in plant sources. The phytate molecule is myo-inositol 1,2,3,4,5,6-hexakis (dihydrogen phosphate) and is primarily present as a salt of mono- and divalent cations (Reddy, 1982).

Chickens have a very limited ability to utilize phytate P because they lack the enzyme phytase, which catalyzes phytate P hydrolysis. This form of P accounts for roughly two-thirds of the P in plants, therefore the available P in plants is approximately 30% of the total P in the plant. However, there has been a long dispute concerning the availability of dietary phytate P to poultry. Gillis et al. (1957) reported that young chicks utilized ^{32}P labeled calcium phytate about one-tenth as effectively as inorganic phosphate. Waldroup et al. (1964) did a comparison of phytin P sources and their ability to support chick growth and tibia ash. They concluded that P from phytic acid was highly available and equal to that of feed grade dicalcium phosphate. Sodium phytate was less available than phytic acid P, and calcium phytate P was relatively unavailable for chick growth. These data were supported by results of an experiment reported by Salman and McGinnis (1968) in which laying hens were fed 0.6% plant P versus 0.3% plant P + 0.3% inorganic P.

Table 1. Comparative Biological Value of Inorganic Phosphates (Scott et al., 1976)

	Biological Value[a]
Reagent grade orthophosphates	
Beta-tricalcium phosphate (anhydrous)	100
Dicalcium phosphate (hydrated)	100
Dicalcium phosphate (anhydrous)	90
Monocalcium phosphate (anhydrous)	113
Potassium phosphate monobasic (anhydrous)	109
Sodium phosphate monobasic (hydrated)	103
Feed grade phosphates	
Dicalcium phosphate	97
Dicalcium/monocalcium phosphate	105-110
Defluorinated phosphates	
Calcified	94
Fused	82
Precipitated	99
Raw rock phosphates	
Curacao Island phosphate	50-87
Tennessee brown rock	**
Colloidal phosphate	**
Bone products	
Steamed bone meal	90-100
Foreign bone meal	87
Spent bone meal	84
Bone ash	89
Pyrophosphates	
Calcium pyrophosphate	00
Calcium acid pyrophosphate	60
Metaphosphates	
Calcium metaphosphate	00
Vitraeous calcium metaphosphate	45
Sodium metaphosphate	00
Calcium phytate	00

[a]Values based on bone calcification in chicks from each source compared with beta-tricalcium phosphate, which was assigned a value of 100.

The hens fed 0.3% plant P + 0.3% inorganic P had a slightly higher rate of lay than hens fed 0.6% plant P, but the difference was not significant. No other symptoms of a P deficiency were detected in the hens fed only plant P, so the authors concluded that hens could meet all their P requirement from plant P.

Singsen et al. (1969) conducted an experiment with caged layers comparing the availability of phytate P with several other P sources. They reported that body weight gain of hens fed yellow corn P or colloidal (soft) P was less than weight gained by hens fed defluorinated rock phosphate. Hens fed Curacao rock phosphate had an intermediate level of weight gain. Egg production was not influenced by P source, but mortality was consistently higher when hens received yellow corn or Curacao rock phosphate as their P source. Watts and Miner (1959) reported apparent digestibilities of 46.6% for P from dicalcium phosphate versus 32.2% for P from colloidal (soft) phosphate.

A recent study (Anonymous 1984) reported that chicks utilized only 7% of the phytate P in their foodstuffs. This is in relative agreement with the findings of Nelson (1976) who reported 0 to 13% phytate hydrolysis in chicks and laying hens. The low values for phytate P hydrolysis in poultry reported by (Anonymous 1984) and Nelson (1976) do not support the earlier work done by Waldroup et al. (1964), and Salman and McGinnis (1968) in which phytate P sources adequately supported chick growth and egg production, respectively. The explanation of why such diverse results have been reported on the utilization of phytate P may be

due to a wide range of factors which can affect phytate P bioavailability and the varying conditions of each experiment.

Factors affecting phytate-P bioavailability

An extensive review of factors affecting phytate P utilization was published in 1967 by Nelson. Some of the factors include dietary calcium, phosphorus, fiber, age of the bird, and the feed source of phytate.

Calcium Numerous experiments with various species have been reported concerning the adverse effects of dietary Ca on phytate P utilization (Nahapetian and Young, 1980; Wise, 1983; Ballam et al., 1984 and 1985). Wise (1983) described the formation of a calcium-phytate chelate in the small intestine which inhibits phytate P hydrolysis. According to Wise (1983), the ratio of dietary Ca to phytate was the primary factor determining the fate of phytate in the intestine. A ratio of 6:1, or greater, enhanced the formation of the relatively insoluble calcium-phytate chelate. In poultry diets, Ca to phytate ratios often approach the range of 6:1, dependent on which grain sources are being utilized. An explanation of the decreased phytate P utilization when dietary Ca level is increased in poultry diets could be the formation of a calcium-phytate complex.

Fiber The effect of fiber sources on phytate P utilization is not clearly defined. Ballam et al. (1984) fed broiler chicks a variety of fiber sources: rice bran, wheat bran, alfalfa meal, cellulose or cottonseed meal. Phytate hydrolysis was greater in chicks fed alfalfa meal and cellulose (25.3 and 31.6%) than in chicks fed rice bran (13.0%

hydrolysis), wheat bran (16.6%), and cottonseed hulls (6.7%). The difference in phytate hydrolysis of different fiber sources may have been the result of different types and levels of fiber in each feed source. Wise (1983) suggested that fiber may be influencing the number and metabolic activities of bacteria in the rat cecum which, in turn, may have a secondary effect on phytate hydrolysis.

Age Nelson (1967) suggested that the utilization of phytate P by poultry increases with increasing age up to maturity. However, in an experiment measuring phytate hydrolysis in broiler chicks versus laying hens, Nelson (1967) found little difference in the ability of hens to utilize phytate P as compared with the chicks. Possibly, gut microflora adapt to and hydrolyze more phytate P as animals age (Wise, 1983).

Phosphorus The amount of available dietary P (non-phytate P) will also influence phytate P utilization. Ballam et al. (1985) conducted a 2 X 2 factorial experiment in which chicks were fed 0.09% or 1.0% Ca and 0.12 or .45% non-phytate P. They reported that increasing the non-phytate P to 0.45% improved phytate hydrolysis in the 0.09% Ca diets; however, when chicks were fed 1.0% Ca the additional P decreased phytate hydrolysis by chicks. Similar results were reported by Ballam et al. (1984). These data imply that the effect of dietary nonphytate P on phytate P hydrolysis is modified by the level of dietary Ca.

Feed source Total phytate P and phytase activity will vary among feed sources and can influence the bioavailability of phytate P. In an extensive study in China, various feedstuffs were analyzed for their phytic acid content. They found that phytate P comprised the following

proportions of the TP in these foodstuffs: 48 to 73% for cereals (corn, barley, rye, wheat, rice, sorghum); 48 to 79% for brans (rice, wheat); 27 to 41% for legume seeds (soybeans, peas, broad beans); 40 to 65% for oil-seed meals (soybean meal, cottonseed meal, rapeseed meal). Some feedstuffs may also have some phytase present, which when ingested by a bird, could catalyze hydrolysis of phytate P in the gastrointestinal tract. Plant phytase activities have been detected in sun-cured alfalfa meal, wheat, and barley mea

SECTION I. EFFECTS OF CALCIUM AND PHASE FEEDING PHOSPHORUS ON PRODUCTION TRAITS AND PHOSPHORUS RETENTION IN TWO STRAINS OF LAYING HENS

ABSTRACT

A 336-day experiment was conducted to test the effects of dietary calcium (Ca) on the adequacy of different total phosphorus (P) feeding programs for two strains of Single Comb White Leghorn hens. Three levels of dietary Ca (3.0, 3.5, or 4.0%) were fed throughout the experiment in a complete factorial arrangement with three P programs. One P program involved feeding .64% phosphorus (P) continuously. Other P programs tested were .56, .49, and .39% P or .64, .54, and .44% P fed when hens were 24 to 36 (phase 1), 36 to 52 (phase 2), and 52 to 72 (phase 3) weeks of age, respectively. In addition to production traits, measurements of P retention were made at 34, 42, 50. 62, and 72 weeks of age. No dietary effects were observed on egg production, egg weight, shell thickness, feed intake, and serum calcium. However, total femur ash (g) and serum inorganic P were significantly greater in hens fed higher levels of dietary P. An average P retention over the entire experiment, on the basis of weighted means of measurements taken five times during the trial, was 104, 148, and 178 mg/day when hens were fed 3.0, 3.5, and 4.0% Ca, respectively. Most of the favorable effect of Ca on P retention occurred after 50 weeks of age. However, increasing dietary Ca had an adverse influence on P retention at 42 weeks. At this time, ambient temperature was unusually hot, and feed intake decreased markedly.

Thus, it seems that ambient temperature may have influenced the effect of dietary Ca on P retention during this time. P feeding

programs had an inconsistent effect on P retention during the experiment, with significant effects observed when hens were 42 and 72 weeks of age. At these times, hens fed .64% P retained the most P while those phase fed .56, .49, and .39% P retained the least. An interaction effect of Ca and P on P retention was present at 62 and 72 weeks of age, with hens fed .64% P and 4.0% Ca retaining less P as compared to hens being phase fed P and 4.0% Ca. The favorable effects of high Ca and P retention at these times, however, was greatest when .49 and.39% P was fed. P retention tended to decline with increasing age of the hens. Overall, the hens were in a state of marginal to negative P balance at 42, 62, and 72 weeks of age when P deposited in eggs was considered.

INTRODUCTION

The National Research Council (NRC, 1984) recommends feeding .32% available (non-phytate) phosphorus (AP) (350 mg/hen daily) continuously through the laying cycle. The use of phosphorus (P) phase-feeding programs for laying hens has been receiving attention recently. AP phase-feeding programs adjusts dietary P according to the stage of production or age of the laying hen. Harms (1979) recommended that hens be fed 650, 550, and 450 mg total P/hen daily for the age periods of 22-36, 36-52, and 52 weeks to end of lay, respectively. Mikaelin and Sell (1981) reported that, if daily feed consumption was 95 to 100 g/hen, then phase-feeding of AP at levels of .46, .36, and .26% and possibly, .36, .26, and .15% during age intervals of 24 to 39, 39 to 51, and 51 to 71 weeks, respectively, would support high rates of egg production without excessively depleting the hen's P stores. In comparison with feeding .45% AP continuously, Rodrigez et al. (1984) reported an improvement in feed utilization when AP levels of .32, .25, and .15% were phase-fed during age intervals of 22 to 34, 34 to 50, and 50 to 70 weeks, respectively. Rodriguez et al. (1984) also observed that hens receiving the P phase-feeding program weighed less and had significantly less carcass ash and P levels than did hens fed .45% AP continuously.

Past research has shown various effects of dietary P and Ca on P retention. Summers et al. (1976) reported that dietary concentrations of Ca and P had little or no effect on P retention of laying hens. Hurwitz and Griminger (1962) reported that P excretion decreased linearly as dietary P decreased; however total P retention (g/hen daily) increased as

dietary P increased from .13 to .51%.

The objective of the research reported here was to test the adequacy of two P feeding programs for egg production and retention of P by laying hens. Also, the influence of different levels of Ca on production characteristics and P utilization was measured.

MATERIALS AND METHODS

Three hundred and sixty Single Comb White Leghorn hens of two commercial strains (144 Dekalb XL [Strain A] and 216 Hyline W-36 [Strain B]) were used. The hens were identical in age and had been reared under the same management system. The hens were housed in wire cages (25 x 40 x 44 cm), two hens per cage. Feed and water were provided ad libitum during the experiment. The experiment began when the hens were 24 weeks of age and at a level of 75% egg production. From 18 to 24 weeks of age, the hens were fed a corn-soybean meal layer diet with .55% total P and 3.4% Ca. The hens were randomly assigned to the experimental units.

Three levels of dietary Ca (3.0, 3.5, or 4.0%) were fed throughout the experiment in a complete factorial arrangement with three P feeding programs. One P program involved feeding .64% P (.40% AP) continuously during the experiment. Other P programs tested were .64, .54, and .44% P (.40, .30, and .20% AP) or .56, .49, and .39% P (.34, .25, and .15% AP), which were fed when hens were 24 to 36 (phase 1), 36 to 52 (phase 2), and 52 to 72 (phase 3) weeks of age, respectively. Each of the nine diets was assigned to five experimental units. Two experimental units within each treatment were assigned hens of the Dekalb XL strain, while the remaining three units in each treatment group were assigned Hyline W-36 hens. Each experimental unit was four consecutive cages with two hens per cage. The corn-soybean diets were isonitrogenous and isocaloric, and dicalcium phosphate was used as the major source of inorganic P. The diets were fed during Phase 2 are representative of those used during the experiment (Table 1). Calculated values of dietary P and Ca were used in

Table 1. Composition of the experimental diets used for phase 2 (weeks 36-52)

% Phosphorus	.64			.54			.49		
% Calcium	3.0	3.5	4.0	3.0	3.5	4.0	3.0	3.5	4.0
Ingredient:	(% of ration)								
Ground Yellow Corn	67.13	64.28	61.43	67.63	64.77	61.92	67.87	65.01	62.17
Soybean Meal (47.0%)	22.88	23.36	23.82	22.79	23.27	23.74	22.75	23.23	23.69
Ground Limestone	6.72	8.02	9.34	7.03	8.34	9.65	7.19	8.50	9.81
Dicalcium Phosphate	1.68	1.69	1.70	1.14	1.15	1.16	.87	.88	.89
Fat	.69	1.75	2.81	.51	1.57	2.63	.42	1.48	2.54
DL-Methionine	.10	.10	.10	.10	.10	.10	.10	.10	.10
Vitamin Premix[a]	.50	.50	.50	.50	.50	.50	.50	.50	.50
Mineral Premix[b]	.30	.30	.30	.30	.30	.30	.30	.30	.30
Calculated Analysis									
Metabolizable energy, kcal/kg	2920.00	2920.00	2920.00	2920.00	2920.00	2920.00	2920.00	2920.00	2920.00
Protein %	16.00	16.00	16.00	16.00	16.00	16.00	16.00	16.00	16.00
Calcium %	3.00	3.50	4.00	3.00	3.50	4.00	3.00	3.50	4.00
Phosphorus, available %	.40	.39	.40	.30	.30	.30	.25	.25	.25
Phosphorus, total, %	.64	.64	.63	.54	.54	.54	.49	.49	.49
Lysine, %	.82	.83	.84	.82	.83	.84	.82	.83	.84
TSAA, %	.60	.60	.60	.60	.60	.60	.60	.60	.60

[a]Contributed the following per kilogram of diet: vitamin A, 4000 I.U.; vitamin D_3, 1500 I.U.; vitamin B_{12}, 5ug; riboflavin, 3.5 mg; Ca. pantothenate, 6.0 mg; niacin, 22 mg; choline, 100 mg.

[b]Contributed per kilogram of diet: manganese, 20 mg; sodium chloride, 2.95g.; and iodine .23 mg.

the tables, laboratory analysis of dietary P and Ca were performed periodically and were consistent with the calculated values. The limestone sieve analysis yielded 6% passing through a 200 mesh screen.

The experiment consisted of 12 periods of 28 days each. Egg production and mortality were recorded daily. Body weights were determined at the beginning of the experiment and at the end of each phase. Feed consumption was recorded for each 28-day period. Eggs produced during the last 2 days of each period were weighed, and shell thickness was measured from periods 5 through 12. Shell thickness was determined by average micrometer readings taken at three points around the equator of each egg.

P retention was determined when the hens were 34, 42, 50, 62, and 72 weeks of age. For this purpose, one bird (chosen at random) from each experimental unit (five/diet) was transferred to an individual pen equipped with an excreta-collection tray. The hens were given a 1-week adjustment period. Chromic oxide was included in the diets at a level of .3% to function as a non-absorbable marker. After the adjustment period, the diets were fed to the hens for 1 week, and excreta were collected during the last 3 days of this week. Diet and excreta were analyzed for P and chromic oxide. Chromic oxide was determined spectrophotometrically by using the procedure described by Fenton and Fenton (1979). P was determined by a colorimetric auto-analyzer procedure according to the Association of Official Analytical Chemists methods (1980). Values of P content obtained from laboratory analysis of the diets and excreta were

used to calculate P retention.

At the completion of the experiment, blood samples and one femur were collected from one bird in each experimental unit. Blood was obtained by heart puncture post oviposition between 1300 and 1500 hrs, (lights on at 0600 hrs). Serum was analyzed for Ca and inorganic P spectrophotometrically using a Ca Rapid-Stat kit and a P AUTO/STAT Kit (Lancer Chemistries and Analytical Systems 1149 Chess Drive, P. O. Box 8029, Foster City, CA 94404). The P Auto/Stat Kit utilizes molybdenum to complex with inorganic orthophosphates, which forms phosphomolybdic acid. The phosphomolybdic acid is then reduced by weak reductants to give a molybdenum blue solution which is measured colorimetrically. The procedure follows Beer's Law over the range 0 to 15 mg P/dl. The femurs were cleaned, fat extracted and ashed at 600^{0} C overnight.

The data were analyzed by using the Statistical Analysis System (SAS) developed by the SAS Institute, Raleigh, NC (Barr et al. 1979). The general linear models procedure was used to test for main effects and interactions.

RESULTS AND DISCUSSION

None of the production characteristics (hen-day egg production, average egg weight, shell thickness, or feed intake) was affected significantly ($p>.05$) by dietary P or Ca over the 336 day experiment (Table 2). However, egg production significantly increased as dietary Ca increased from 3.0 to 3.5 and 4.0% during phase 3 of production (Table 3). Significant strain effects were detected. Hens of strain A produced eggs that were heavier ($p<.05$) during Phase 1 and had thinner shells ($p<.08$) than those of strain B (Table 3 and 4). Strain A hens also consumed less feed ($p<.01$) over the entire experiment. Diet treatment x strain interaction effects on production traits were observed in two instances. The significant Ca x P x strain effect ($p<.006$) was the result of a favorable influence of dietary Ca level on rate of egg production in only one strain of hens during phase 2. A P x strain interaction effect on shell thickness was indicated ($p<.07$) and was the result of shell thickness being greater for one strain of hens than for the other when decreasing amounts of P were fed.

Hens phase-fed diets containing .56, .49, and .39% P during intervals 24 to 36, 36 to 52, and 52 to 72 weeks, respectively, consumed an average of 614, 565, and 405 mg P/hen daily during phases 1, 2, and 3, respectively (Table 3). In contrast, hens fed .64% P continuously consumed 676 mg P (423 mg AP)/hen daily. The production data demonstrate that the lower P intakes of phase-fed hens were adequate to prevent overt signs of deficiency, irrespective of dietary Ca level. The results of the experiment reported herein agree with those of a number of previous

Table 2. Influence of dietary phosphorus, calcium and strain on egg production, egg weight, shell thickness, feed intake, phosphorus and calcium intake, femur ash, and serum phosphorus and calcium

Dietary Treatment										
Phosphorus[a] %	Calcium %	Egg Prod. %	Egg Weight (g)	Shell Thickness (mm)	Feed Intake (g/hen/ /day)	P Intake (mg/hen/ /day)[b]	Calcium Intake (g/hen/ day)	Femur Ash (g)[c]	Serum Inorganic P (mg/dl)	Serum Calcium (mg/dl)
.64	3.0	80.0	59.5	.394	106	679	3.19	2.72	8.04	33.5
.64	3.5	81.8	59.7	.393	106	681	3.72	2.70	7.45	30.3
.64	4.0	82.4	59.1	.393	104	669	4.18	2.86	8.02	33.5
.64-.54-.44	3.0	81.8	59.3	.390	106	574	3.19	2.41	5.89	28.8
.64-.54-.44	3.5	83.6	58.5	.395	106	569	3.70	2.81	6.74	32.6
.64-.54-.44	4.0	82.4	60.1	.403	104	564	4.17	2.66	6.84	34.2
.56-.49-.39	3.0	78.1	58.6	.400	102	491	3.06	2.36	5.67	33.2
.56-.49-.39	3.5	82.2	59.2	.392	105	503	3.67	2.41	6.10	28.0
.56-.49-.39	4.0	82.3	59.0	.402	104	499	4.18	2.54	6.51	36.2
S.E.M.[d]		2.0	68	.007	2.0	054	.31	.16	.79	2.9
Source of Variation							Probabilities			
Calcium (Ca)		NS	NS	NS	NS	NS	.0001	NS	NS	NS
Phosphorus (P)		NS	NS	NS	NS	.0001	NS	.05	.0001	NS
Ca x P		NS	NS	NS	NS	NS	NS	NS	NS	NS
Strain		NS	.05	.0	.01	.01	.01	.08	.0003	.02
Ca x Strain		NS	NS	NS	NS	NS	NS	NS	NS	NS
P x Strain		NS	NS	.07	NS	NS	NS	NS	NS	NS
Ca x P x Strain		.02	NS	NS	NS	NS	NS	NS	NS	NS

[a]dietary concentrations of total phosphorus fed continuously or during age periods of 24 to 36, 36 to 52, and 52 to 72 weeks, respectively.

[b]Average phosphorus intake based on average feed intake during the entire experiment.

[c]Femur ash calculated as grams ash/1 femur bone.

[d]Standard error of the means.

Table 3. Influence of dietary phosphorus, calcium, and strain on phosphorus intake, egg production and egg weight for Phase 1, 2, and 3

Dietary Treatment		Phase 1			Phase 2			Phase 3		
Phosphorus %	Calcium %	P Intake (mg/hen day)	Egg Production (%)	Egg Weight (g)	P Intake (mg/hen day)	Egg Production (%)	Egg Weight (g)	P Intake (mg/hen day)	Egg Production (%)	Egg weight (g)
.64	3.0	649	88.0	56.5	714	77.0	58.6	785	73.2	62.5
.64	3.5	658	90.2	57.1	711	78.6	59.2	797	78.6	62.4
.64	4.0	635	88.4	56.3	680	76.0	57.8	800	81.0	62.2
.64-.54-.44	3.0	657	89.0	56.8	682	81.8	58.6	539	74.8	61.5
.64-.54-.44	3.5	645	91.4	55.3	652	79.0	57.9	561	78.0	61.2
.64-.54-.44	4.0	666	92.0	57.4	635	75.8	59.4	542	78.6	62.3
.56-.49-.39	3.0	614	90.8	55.9	554	75.4	58.3	438	68.8	60.8
.56-.49-.39	3.5	617	86.8	56.2	586	84.6	58.5	445	73.8	61.5
.56-.49-.39	4.0	612	91.4	55.1	556	75.8	58.5	469	80.6	62.3
Strain A		656	90.5	57.6	659	79.5	59.1	595	76.5	62.1
Strain B		628	89.2	55.3	628	77.3	58.1	599	76.3	61.7
S.E.M.[a]		2.1	1.8	.77	6.2	3.2	.51	15.4	4.0	.62
Source of Variation		Probabilities								
Calcium		NS	NS	NS	NS	NS	NS	NS	.005	NS
Phosphorus		.0001	NS	NS	.0001	NS	NS	.0001	NS	NS
Ca X P		NS	NS	NS	NS	NS	NS	NS	NS	NS
Strain		.0002	NS	.0001	.02	NS	NS	NS	NS	NS
Ca X Strain		NS	NS	NS	NS	NS	NS	NS	NS	NS
P X Strain		NS	NS	NS	NS	NS	NS	NS	NS	NS
Ca X P X Strain		NS	NS	NS	NS	.006	NS	NS	NS	NS

[a]Standard error of the means.

Table 4. Summation of data by strain of hen for the entire experiment

Variable	Strain A	Strain B
Egg Production (%)	82.2	81.0
Egg Weight (g)**	60.0	58.7
Shell Thickness (mm)*	.391	.399
Feed Intake (g/day)***	107.1	102.8
Average Phosphorus Intake (g/day)[a]***	594	568
Calcium Intake (g/day)***	3.75	3.60
Femur Ash (g)[b]*	2.70	2.51
Serum Phosphorus (mg/dl)***	6.21	7.40
Serum Calcium (mg/dl)**	30.0	34.5
P Retention - 34 Weeks (mg/day)*	212	306
P Retention - 42 Weeks (mg/day)	53	83
P Retention - 50 Weeks (mg/day)	156	145
P Retention - 62 Weeks (mg/day)	66	71
P Retention - 72 Weeks (mg/day)	94	61
Average P Retention - (mg/day)	134	154

[a]Quantities per day are on a per hen basis.

[b]Femur ash calculated as grams ash/1 femur bone.

*Significant Strain Effect at $p<.10$.

**Significant Strain Effect at $p<.05$.

***Significant Strain Effect at $p<.01$.

studies on phase-feeding programs. Mikaelian and Sell (1981) conducted two long-term experiments (44 weeks of production) and, in one, found that hens phase-fed P maintained satisfactory production characteristics while consuming as little as 155 mg AP/hen daily. The results of a second experiment, however, indicated that 252 mg AP/hen daily was barely adequate. Rodriguez et al. (1984) also tested P phase feeding programs and found that hens fed .35, .25, and .15% AP from 22 to 34, 34 to 50, and 50 to 70 weeks of age, respectively, consumed an average of 250 mg AP/hen daily and produced eggs at a rapid efficient rate. This was true for each of three commercial strains of hens used in the experiment. In the same experiment, egg production was inferior when .15% AP was fed continuously.

Dietary P significantly affected femur ash (g ash/femur) ($p<.05$) and serum P ($p<.0001$) (Table 2). Hens fed .64% P continuously, had 2.76 g ash/femur and 7.84 mg/dl serum inorganic P, whereas hens phase-fed .64, .54, and .44% P and .56, .49, and .39% P had 2.63 and 2.44 g ash/femur and 6.49 and 6.09 mg/dl serum P, respectively. Serum samples were collected several hours post-oviposition; therefore, the serum P levels are indicative of P status during the non-calcifying stage of egg production. At this time, serum P decreased as the dietary P decreased indicating a dietary inadequacy of P to support high serum inorganic P levels. This dietary inadequacy is also shown by the femur ash levels. Femur ash decreased as dietary P decreased indicating an insufficient supply of dietary P to prevent bone demineralization. The favorable

effects of increasing dietary P on bone ash were not observed by Mikaelian and Sell (1981) or Owings et al. (1977), who detected no significant differences in tibia or femur ash as dietary P varied. The detection of a significant dietary P effect on femur ash in the current experiment may have been facilitated by the large number of replicates and the long duration of the experiment as compared with the studies of Mikaelian and Sell (1981) and Owings et al. 1977).

Significant strain effects on femur ash ($p<.08$), serum P ($p<.0003$), and serum Ca ($p<.02$) were observed at the conclusion of the experiment (Table 4). Femur ash was greater in strain A, whereas serum P and Ca concentrations were greater in strain B. These data seem inconsistent with the feed consumption data showing lower P and Ca intake by strain B. One would expect serum P and Ca to increase as dietary intakes of P and Ca increase, whereas just the opposite effect occurred with strain B. But serum P and Ca were measured at the end of the experiment, whereas P and Ca consumption data were a compilation of measurements taken at 4-week intervals.

Phosphorus retention (mg P retained/day) was measured at 34, 42, 50, 62, and 72 weeks of age. Phosphorus retention did not include a calculation of P loss in eggs produced. Treatment means for P consumption and P retention during these collection trials are presented in Table 5. Means showing main effects and interactions of dietary Ca and P are given in Table 6. Egg production was 78, 83, 76, and 74% during the 34, 42, 50, and 62 weeks of age collection trials, respectively; however, no dietary treatment effects were detected on egg production at these times.

Table 5. Influence of dietary phosphorus, calcium, and strain on phosphorus consumption and retention[a]

Dietary Treatment		34 Weeks		42 Weeks		50 Weeks		62 Weeks		72 Weeks	
P (%)	Ca (%)	P Intake[b]	P Retention[c]	P Intake	P Retention	P Intake	P Retention	P Intake	P Retention	P Intake	P Retention
.64	3.0	831	92	817	149	813	39	767	136	722	85
.64	3.5	802	204	727	163	838	107	788	19	755	204
.64	4.0	915	306	801	47	795	228	790	54	736	46
.64-.54-.44	3.0	932	295	647	132	687	45	589	121	546	81
.64-.54-.44	3.5	882	326	561	28	734	175	491	115	429	22
.64-.54-.44	4.0	763	142	596	87	719	263	506	159	510	145
.56-.49-.39	3.0	826	230	521	7	629	221	387	7	413	35
.56-.49-.39	3.5	944	409	622	126	681	158	491	39	456	2
.56-.49-.39	4.0	811	375	578	13	663	189	531	135	473	116
S.E.M.[d]			76		41		63		44		30
Source of Variation:											
Calcium (Ca)			NS		NS		.02		.06		.06
Phosphorus (P)			NS		.07		NS		NS		.01
Ca x P			NS		.01		NS		.01		.001
Strain			.10		NAS		NS		NS		NS
Ca x Strain			NS		NS		NS		NS		NS
P x Strain			NS		NS		NS		NS		NS
Ca x P x Strain			NS		NS		NS		NS		NS

[a]Retention concentrations of total phosphorus fed continuously or during age periods of 24 to 36, 36 to 52, and 52 to 72 weeks, respective.

[b]Phosphorus intake during the collection trail period (mg/hen/day)

[c]P retention, mg/hen daily = P consumed, mg/hen daily - P excreted, mg/hen daily.

[d]Standard error of the means.

Table 6. Main effects of calcium and dietary phosphorus on phosphorus retention

Age of Hens at Measurement of Phosphorus Retention	Phosphorus Retention, mg P Retained/Hen Daily					
	Calcium, %			Phosphorus Feeding Program, %		
	3.0	3.5	4.0	.64 Cont.	.64-.54-.44	.56-.49-.39
Week 34	205	321	284	200	263	338
Week 42[a]	96	78	40	117	64	34
Week 50[b]	76	145	226	99	160	189
Week 62[c]	27	58	120	71	77	60
Week 72[d]	41	76	107	117	83	28
Average	104	158	178	135	157	149

[a]Significant P main effect at $p<.07$.

[b]Significant Ca main effect at $p<.02$.

[c]Significant Ca main effect at $p<.06$.

[d]Significant Ca main effect at $p<.06$ and P main effect at $p<.05$.

Average P retention was 269 mg/day at 34 weeks of age; thereafter, the retention decreased to 72, 149, 69, and 75 mg/day at 42, 50, 62, and 72 weeks of age, respectively. At 34 and 50 weeks of age, the hens were in a state of positive P balance even if P loss in the egg were considered. However, when egg P loss was estimated at 80-120 mg/egg, the hens would have been in a marginal to negative state of P balance at 42, 62, and 72 weeks of age. A negative P balance was also reported by Hurwitz and Griminger (1962) in hens consuming less than 240 mg P/day. Only a slight strain effect ($p<.10$) on P retention was observed at 34 weeks, with strain B retaining 306 mg/day while strain A retained 212 mg/day.

During the collection period at 42 weeks of age, the average house temperature rose to 30°C (Figure 1). A corresponding decline in average P retention to 71 mg/day also occurred at this time. It is most likely that the decline in P retention was a result of a decrease in feed consumption due to the high house temperatures. Charles et al. (1978) found that the laying hen had a higher percentage dietary requirement for P during hot temperatures. Garlich (1979) reported increased mortality during high temperatures in hens fed low P diets. The data presented herein did not show a decrease in P retention when hens were fed lower levels of P during high house temperatures. Furthermore, significant P ($p<.07$) and Ca X P ($p<.01$) effects of P retention were observed during this time. When dietary P increased from .49 to .54 and .64%, P retention increased from 34 to 64 and 117 mg/day. Although the main effect of Ca was not significant in this instance, P retention decreased

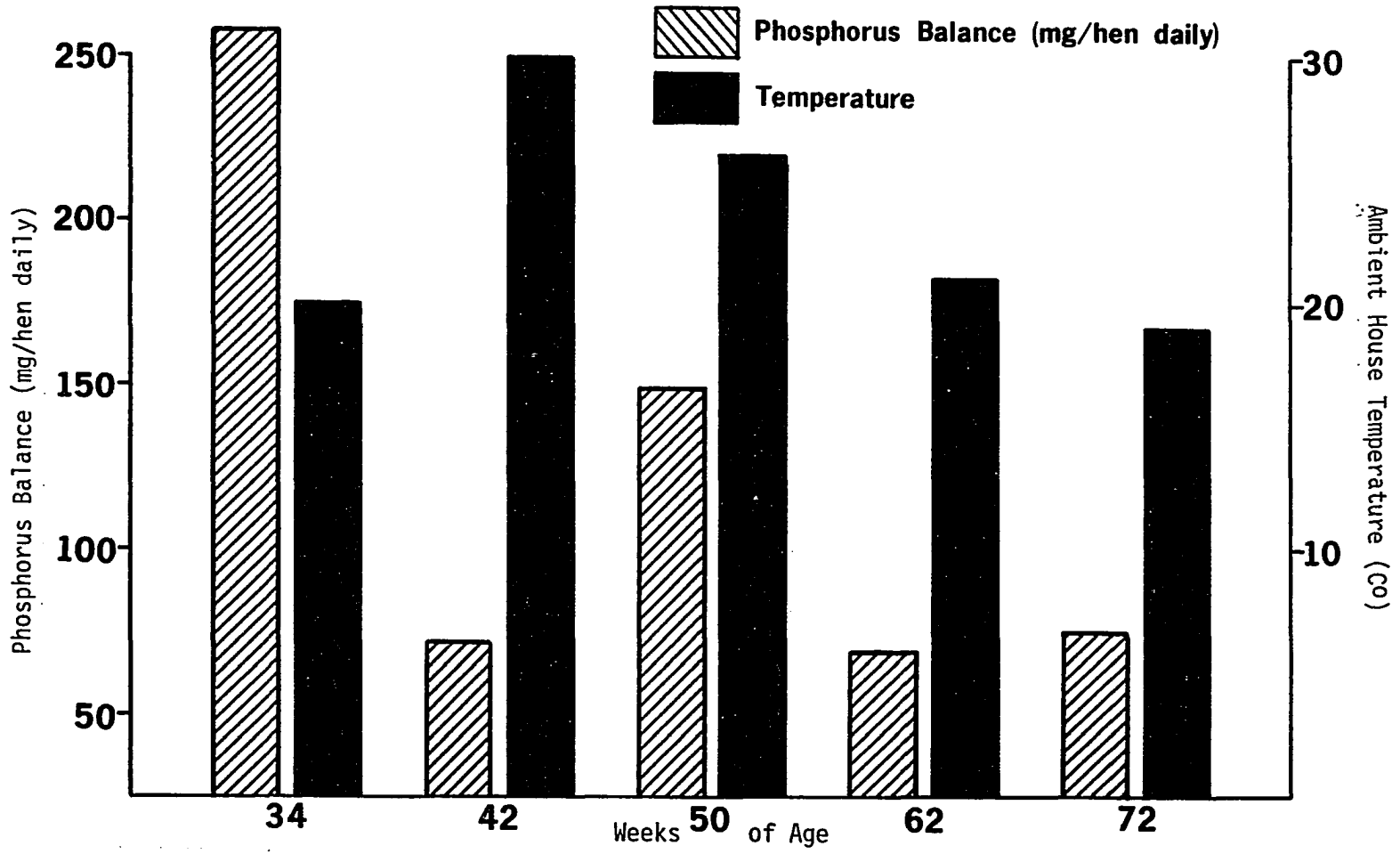

Figure 1. Average Phosphorus Retention by age and temperature

as dietary Ca increased from 3.0 to 3.5 and 4.0%.

A detrimental effect of increasing dietary Ca on P retention was seen only at 42 weeks of age. At 50, 62, and 72 weeks of age, P retention increased significantly as dietary Ca increased from 3.0 to 4.0%. The weighted mean for the entire experiment also showed P retention increasing as dietary Ca increased. At 50, 62, and 72 weeks of age, the house temperatures were moderate (Figure 1) compared with week 42. These data suggest that temperature influenced the effects of dietary Ca on P retention at 42 weeks of age.

At 62 weeks of age, a significant Ca x P interaction effect ($p<.01$) was detected. Hens continuously fed .64% P, had a greater P retention when fed 3.0% Ca versus 3.5 and 4.0% Ca. The opposite effect of dietary Ca was observed in hens phase-fed .44 and .39% P. In the latter instance, P retention increased as dietary Ca increased. This is in accord with the beneficial effect of increasing dietary Ca on P retention.

Data obtained during the final collection period at 72 weeks of age showed significant P ($p<.06$) and Ca x P ($p<.001$) effects. Hens fed .64% P for the entire experiment retained more P than did hens fed .44 and .39% P at the end of their phase feeding programs. P retention was highest in hens fed .64% P and 3.5% Ca (204 mg/day); otherwise, P retention was greatest for phase-fed hens when the diet contained 4.0% dietary Ca.

To illustrate the apparent relationship between P retention and age

of hen, retentions at each determination time, averaged across diet treatments and strain of hens, are shown in Figure 1. Except for the 42-weeks determination time, P retention declined with age through 62 weeks and then seemed to plateau. The relatively low P retention at 42 week coincided with a period of very warm ambient temperature. Average daily high temperatures during each determination time are also shown in Figure 1. In addition to the relatively warm daytime temperatures during week 42, night time temperature and relative humidity remained high. During this interval, feed (and P) intake decreased an average of 16.6%. Undoubtedly, the decrease in P intake and(or) other consequences of heat stress contributed to low P retention at 42 weeks.

At moderate temperatures, P retention increased as dietary Ca increased from 3.0 to 3.5 and 4.0%. The beneficial effect of increasing dietary Ca on P utilization does not agree with results of past research. Wise (1983) reported that an excess of Ca induced the precipitation of a phytate-calcium complex, rendering phytate P less available. Ballam et al. (1984) reported a decrease in phytate P utilization as dietary Ca increased. However, the overall retention of total dietary P increased as dietary Ca increased. Possibly dietary Ca has a different effect on non-phytate and phytate P whereby increasing dietary Ca over a moderate range enhances the utilization of available P while having the opposite effect on phytate P utilization.

The concept of phase-feeding P on the basis of age intervals in laying hens was supported by the results of this experiment. Hens phase-fed maintained adequate rates of egg production, egg weight, and

shell thickness through 48 weeks of production. These data agree with the results of research done by Rodriguez et al. (1984); Said and Sullivan (1982); and Mikaelian and Sell (1981).

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SECTION II. UTILIZATION OF PHYTATE PHOSPHORUS IN POULTRY AS INFLUENCED BY DIETARY CALCIUM AND PHOSPHORUS

ABSTRACT

Two experiments were conducted to measure phytate phosphorus (P) utilization in laying hens, and ^{33}P distribution in chicks as affected by dietary calcium (Ca) and P. Experiment one was a 3 by 3 factorial arrangement of three levels of Ca (3.0, 3.5, and 4.0%) fed with three P feeding programs. The P programs were .64% P fed continously; .64, .54, and .44% P or .56, .49 and .39% P fed when hens were 24-36, 36-52, and 52-72 weeks of age, respectively. Phytate P and % P associated with excreted phytate were measured at 34, 50 and 72 weeks of age. Phytate P was determined colorimetrically and phytate was determined by high-performance liquid chromatography (HPLC). Retention of phytate P was relatively high at 34 weeks of age, averaging 46.6% across diet treatments. Phytate P retention declined to 9.1 and 16.5% at 50 and 72 weeks, respectively. P feeding program altered retention of phytate P at 50 and 72 weeks, but in an inconsistent manner. The influence of Ca on % phytate P retention also varied considerably, but at 50 and 72 weeks of age, as Ca level increased to 4.0%, phytate P retention by laying hens decreased. Furthermore, as Ca increased from 3.0 or 3.5% to 4.0%, the % P associated with phytate in excreta increased. Phytate, in the form of hexaphospho- inositol, should contain 28.4% P by weight. In some instances, hens fed 4.0% Ca in this experiment, excreted phytate that contained greater than 30% P.

Experiment two involved feeding two levels of dietary Ca (.8 and 1.2%) to one-week-old broiler chicks for 7 days during which time 50 uCi ^{33}P/chick was administered. Serum, femurs and excreta were collected 48

hours after ^{33}P dosing. Serum P and excreta ^{33}P activity were greater in chicks fed 0.8% Ca; while femur ^{33}P activity was greater in chicks receiving the 1.2% Ca diet. The %P associated with excreted phytate ranged from 5.6 to 7.0% and phytate P hydrolysis ranged from 31 to 48% The latter values are considerably higher than phytate P hydrolysis in laying hens of experiment one. Excreted phytate also had associated ^{33}P activity after transit through the gut, giving evidence of a phosphate flux between the inorganic ^{33}P pool and the phytate P pool, or the binding of ^{33}P to "free" hydroxyl groups of phytate molecules. The ^{33}P distribution data indicate increased P retention and greater bone P deposition in chicks fed 1.2% dietary Ca.

Overall, the data obtained from this research show that poultry are capable of utilizing phytate P; however, their ability to utilize phytate P changes with age and can be adversely affected by high levels of dietary Ca.

INTRODUCTION

Phytate (inositol hexaphosphate) occurs naturally in many feedstuffs derived from plants. Phytate functions as the primary storage form of both phosphate and inositol in plant seeds. Sources of phytate commonly used for animal production include nearly all cereals, legumes, and oil seeds. The level of phytate varies from source to source. For example, oil seeds (sesame and rapeseed) usually have 1.5% or more phytate, whereas whole-grain cereals (corn, wheat, and rice) have only ca. 1.0% phytate (Erdman, 1979). Hydrolysis of dietary phytate in poultry is dependent on a number of factors, an important one being the presence of phytase. Phytase in not present inherently in the chicken's gastrointestinal tract; however, microflora and some feedstuffs may have phytase activity. Nelson (1967) also reported that the ability of the chicken to hydrolyze phytate P increases with age up to maturity.

Dietary factors also have a significant influence on phytate P utilization in the chicken. Nelson (1976) found that phytate hydrolysis increased in chickens fed wheat as an energy source as compared with corn. Increasing dietary Ca and non-phytate P decreased phytate hydrolysis in chicks, whereas just the opposite effect occurred when low levels of dietary Ca and non-phytate P were fed (Ballam et al., 1981 and 1984).

Wise (1983) concluded that the primary factor determining the rate of phytate hydrolysis is the ratio of dietary Ca to phytate. At high molar concentrations of Ca and a near neutral gastrointestinal pH, Ca binds to phytate, forming a low-solubility chelate. A reduction in

phytate P hydrolysis as dietary intake of calcium increases has been observed in several species of animals (Pointillart et al., 1985, Nahapetian and Young, 1980, Nelson and Kirby, 1979, and Taylor and Coleman, 1979).

Gillis et al. (1957) compared ^{32}P labeled phytate with ^{32}P inorganic P as sources of P for chicks and poults. They found that young chicks utilized ^{32}P labeled calcium phytate only about one-tenth as effectively as inorganic P, and in in vitro studies, an indication of radioactive ^{32}P exchange of inorganic with phytate P was obtained.

The objective of experiment one of the research reported here was to test the effects of dietary Ca and P on phytate P utilization by laying hens and to obtain information on the amount of P bound to phytate in excreta. The second experiment was conducted to determine the effects of dietary Ca on ^{33}P distribution in growing chicks and to gain further information on flux of P on the phytate moiety.

MATERIALS AND METHODS

Experiment One

Three levels of dietary Ca (3.0, 3.5, and 4.0%) were fed for 336 days in a complete factorial arrangement with three P feeding programs. One P program involved feeding .64% P (.40% non-phytate P) continuously during the experiment. Other P programs tested were .64, .54, and .44% P (.40, .30, and .20% non-phytate P) or .56, .49, and .39% P (.34, .25, and .15% non-phytate P), which were fed when hens were 24-36 (phase 1), 36-52 (phase 2), and 52-72 (phase 3) weeks of age, respectively. Each of the nine diets was assigned to five experimental units. Each experimental unit was four consecutive cages with two hens per cage (25 x 40 x 45 cm). Feed and water were provided ad libitum.

The basal diet containing 3.0% calcium and .39% phosphorus is shown in Table 1. Limestone and dicalcium phosphate provided inorganic Ca and P, and their concentrations in diets varied according to dietary treatment. Calculated phytate phosphorus content was .24% in all nine diets during the three phases of the experiment. Calculated dietary P values were used in the results and discussion of this paper. Laboratory analyses of dietary P were performed periodically and the results were consistent with calculated values.

Production traits were recorded throughout the experiment, and these data were reported elsewhere by Scheideler and Sell (1986). Phytate P retention was determined at 34, 50, and 72 weeks of age. For this purpose, one bird from each experimental unit (five/diet) was transferred to an individual pen equipped with an excreta-collection tray. The hens

Table 1. Experiment one basal diet composition and experiment two diet composition

Ingredient	Experiment One Basal Diet	Experiment Two % Calcium .80	Experiment Two % Calcium 1.20
Ground Yellow Corn	67.91	53.23	51.09
Soybean Meal	23.07	36.28	36.49
Meat and Bone Meal		4.00	4.00
Fat	.31	3.73	4.50
Limestone	7.50	.52	1.57
Dicalcium Phosphate	.33	.40	.41
DL-Methionine	.10	.24	.34
Vitamin Premix	.50[a]	.30[b]	.30
Mineral Premix	.30[c]	.30[d]	.30
Phytate[e]		1.00	1.00
Calculated Analysis			
Metabolizable Energy kcal/kg	2920	3100	3098
Protein, %	16.0	23.0	23.0
Calcium, %	3.0	.80	1.20
Phosphorus,available,%	.15	.45	.45
Phosphorus,total, %	.39	.90	.90
Lysine, %	.82	1.33	1.33
TSAA, %	.60	.93	1.03

[a]Contributed per kilogram of diet: vitamin A, 4000 I.U.; vitamin D3, 1500 I.U.; vitamin B12, 5 ug; riboflavin, 3.5 mg; Ca. pantothenate, 6.0 mg; niacin, 22 mg; choline, 100 mg.

[b]Contributed per kilogram of diet: vitamin A, 4500 I.U.; vitamin D3, 1500 I.U.; vitamin E, 6 I.U.; vitamin B12, 10 ug; vitamin K, 1 mg; riboflavin, 3 mg; pantothenic acid, 14 mg; niacin, 75 mg; choline 400 mg.

[c]Contributed per kilogram of diet: manganese, 20 mg; sodium chloride, 2.95 g; and iodine, .23 mg.

[d]Contributed per kilogram of diet: manganese, 70 mg; zinc, 40 mg; iron, 37 mg; copper, 6 mg; selenium, .15 mg; sodium chloride, 2.60 g; and iodine .23 mg.

[e]Sodium phytate salt of inositol hexaphosphoric acid isolated from corn, laboratory analysis of 19% P.

were given a one-week adjustment period. Chromic oxide was included in the diets at a level of 0.3% to function as a nonabsorbable marker. After the adjustment period, the diets were fed to the hens for one week, and excreta were collected during the last three days of that week. Diet and excreta were analyzed for chromic oxide, phytate P, and phytate. Chromic oxide was determined spectrophotometrically by the procedure described by Fenton and Fenton (1979). Feed and excreta samples were prepared for phytate P and phytate analysis by extraction in 3% trichloracetic acid (TCA). An aliquot of the TCA extract was used for detection and quantification of phytate by high-performance liquid chromatography (HPLC) using modified procedures from Tangendjaja et al. (1980) and Knuckles et al. (1982). A Versapack C18 column (300 MM x 4.1mM) (Alltech Associates, Deerfield, IL) was used to chromatograph the samples. Monopotassium phosphate (.025M and pH 6.0 with the addition of NaOH) was used for the mobile phase, followed by a methanol flush. Flow rate was 1.80 ml/min, and average retention time for phytate was 1.6 min. Detection of phytate was done by using a differential refractive index detector (Water Associates, Inc., Milford, MA.) and a chromatopac integrator (Shimadzu Model C-R3A, Kyoto, Japan). Calculation of total phytate includes all forms of inositol phosphate present in the chromatogram. A representative chromatogram of phytate analysis is shown in Figure 1. The remaining TCA extract was used for the determination of phytate P by the procedure reported by Common (1940). Phytate was precipitated with the addition of ferric chloride. The precipitate was wet ashed with nitric, sulfuric, and perchloric acid to liberate phytate

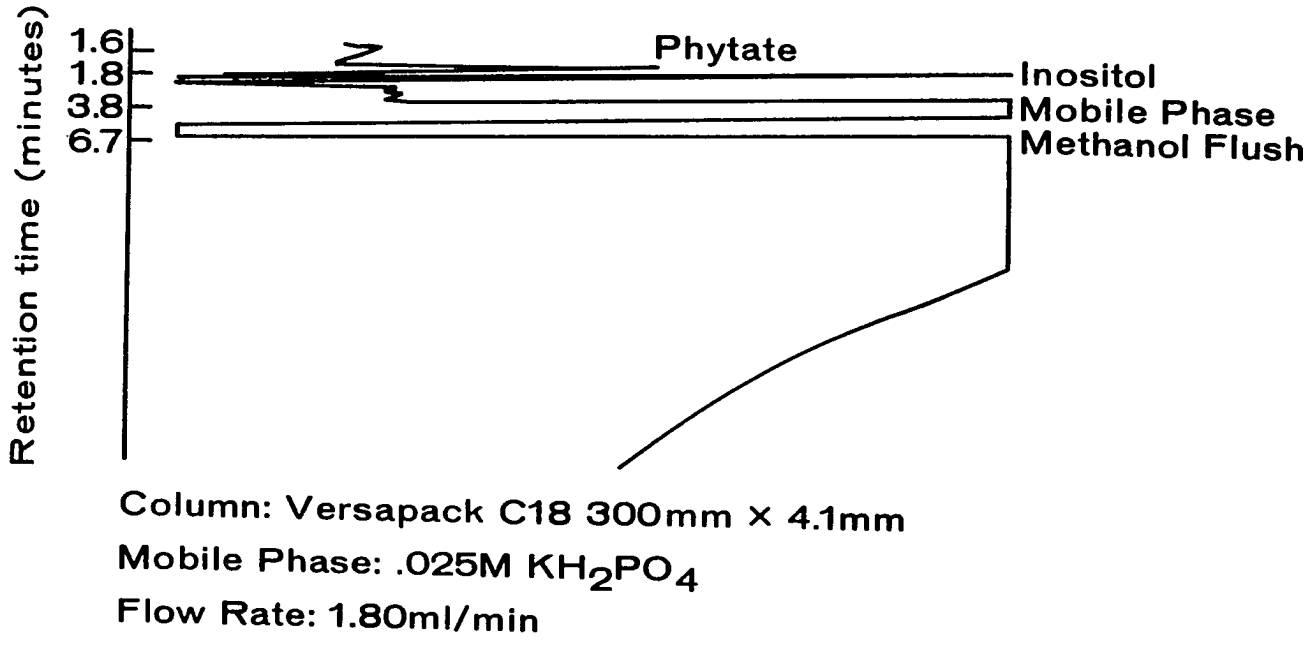


Figure 1. Chromatogram of phytate separation and detection

P. The liberated P was then analyzed colorimetrically after reaction to form a molybdate complex.

Percent phytate P retention was calculated as phytate P consumption minus excreted phytate P divided by dietary phytate P. The % P associated with excreted phytate was calculated as excreted phytate P (determined colorimetrically) divided by excreted phytate (determined by HPLC).

The data were analyzed by using the Statistical Analysis System (SAS) developed by the SAS Institute, Cary, NC. (Barr et al., 1982). The general linear models procedure was used to test for main effects and interactions.

Experiment Two

One-week-old broiler chicks were fed 0.8 or 1.2% Ca diets (Table 1) for one week. Each diet was randomly assigned to eight cages (23cm x 17.5 cm x 15 cm) with two chicks per cage. Feed and water were provided ad libitum. One percent sodium phytate (sodium salt of inositol hexaphosphoric acid isolated from corn (Sigma Chemical Co., St. Louis, Mo.)) was added to each diet. Laboratory analysis of the sodium phytate gave a content of 19% P.

Feed intake was measured daily (g/pen/day) and body weight gain was recorded at the conclusion of the experiment. On day 5, 50 uCi of ^{33}P (sodium monohydrogen phosphate in water (New England Nuclear Products, Boston, MA.)) was given orally in a gelatin capsule to each chick in six of the replicates of each diet. Fecal collection trays were arranged under each cage and the trays were lined with wax paper for excreta

collection. Excreta collections were made 24 and 48 hours after ^{33}P dosing. At 48 hours post ^{33}P dosing, chicks were weighed, blood samples were obtained by heart puncture, and chicks were killed by euthanasia. A femur bone sample was excised from each carcass.

Blood samples were centrifuged and serum was collected. Serum inorganic P was measured by spectrophotometry using a P AUTO/STAT Kit (Lancer Chemistries and Analytical Systems, Foster City, CA.) and serum ^{33}P activity was counted by combining 0.1 ml of serum in a glass scintillation vial with 15 ml Biofluor high efficiency emulsifier cocktail (New England Nuclear, Boston, MA.). Samples were counted for ^{33}P activity on a Packard Tri-Carb 300 liquid scintillation system (Packard Instrument Co., Downers Grove, Ill.). Counting efficiency for ^{33}P exceeded 90% for all tissues counted. Femur bone samples were cleaned, fat extracted, and ashed at 600^{o}C overnight. An aliquot of bone ash was dissolved in dilute HCl. Phosphorus analysis of the aqueous ash was done by a colorimetric auto-analyzer procedure according to the Association of Official Analytical Chemists methods (1980). Bone ^{33}P activity was measured by combining an aliquot of the aqueous ash solution with 10 ml Beckman Ready Solve TM scintillation cocktail (Beckman Instruments, Inc., Fullerton, CA.), and counted by the previously described Packard scintillation counter.

Excreta phytate and phytate P were analyzed by HPLC and colorimetric procedures, respectively, as described in materials and methods section experiment one. Phytate ^{33}P activity was measured by combining a portion of the phytate P preparation with 10 ml Beckman scintillation cocktail and counting in the Packard scintillation counter. The % P associated

with excreted phytate was calculated as described in experiment one. Excreta non-phytate P was determined on the supernatant of the excreta preparation after phytate precipitation (described in experiment one). Non-phytate P was measured colorimetrically and non-phytate ^{33}P activity was measured by combining an aliquot of the supernatant with 10 ml Beckman scintillation cocktail and counting on the Packard scintillation counter.

Excreta phytate ^{33}P and non-phytate ^{33}P activity are expressed as DPM/g excreta dry matter. Excreta phytate P and non-phytate P are expressed on a % basis.

Means were compared by using Student's T-test and data were analyzed by using the Statistical Analysis System (SAS) developed by the SAS Institute, Cary, NC. (Barr et al., 1982).

RESULTS

Experiment One

Numerical values of phytate P retention measured at 34, 50, and 72 weeks of age are shown in Table 2. Data summarized according to main effects of dietary Ca and P are given in Table 3. At 34 weeks of age, % phytate P retention decreased ($p< 0.07$) as dietary Ca increased. Hens retained 52.8, 40.7, and 46.3% phytate P when fed 3.0, 3.5, and 4.0% Ca, respectively. This main effect of dietary Ca was most evident in hens fed the lower P level (.56%) from 24 to 34 weeks. The effect of dietary Ca on % phytate P retention by hens fed .64% P during this time was relatively inconsistent, but neither a Ca X P interaction effect nor a P main effect was detected. Although not significant, % phytate P retention at 72 weeks of age showed a dietary Ca effect with hens retaining 22.5, 16.0 and 11.0% phytate P when fed 3.0, 3.5, and 4.0% Ca, respectively.

Significant P main effects on phytate P retention were observed at 50 ($p<0.06$) and 72 ($p<0.07$) weeks of age. However, this P effect was inconsistent at each time. For example, the main P effect means (Table 3) were 12.6, -.2, and 14.5% for hens fed .64, .54, and .49% P, respectively, to 50 weeks of age while these main effect means were 11.4, 25.0, and 13.2% for hens fed .64, .44, and .39% P, respectively, to 72 weeks. Factors that may have contributed to these inconsistencies cannot be identified from the data obtained. Variation among individual hens on the same treatment with respect to % phytate P retention, resulted in very high standard errors. Also, the ambient temperature varied from one

Table 2. Experiment One: Influence of dietary calcium and phosphorus on % of phytate P retention by laying hens and % phosphorus associated with phytate in excreta

Dietary Treatment		34 Weeks[a]		50 Weeks		72 Weeks	
Phosphorus (%)	Calcium (%)	% Phytate P Retention	% P associated with phytate	% Phytate P Retention	% P associated with phytate	% Phytate P Retention	% P associated with phytate
.64	3.0	48.4	19.7	15.7	17.7	15.5	29.7
.64	3.5	47.4	18.5	6.71	24.6	16.2	25.9
.64	4.0	53.7	19.3	21.54	33.6	2.4	43.8
.64-.54-.44	3.0	57.4	22.0	(-)6.4	16.2	18.3	19.7
.64-.54-.44	3.5	44.0	19.6	12.9	20.1	23.1	19.4
.64-.54-.44	4.0	44.0	29.2	(-)4.4	35.6	33.4	16.0
.56-.49-.39	3.0	52.4	19.6	16.4	28.1	33.8	14.1
.56-.49-.39	3.5	31.9	18.5	20.3	23.0	8.6	17.2
.56-.49-.39	4.0	41.3	25.6	7.0	24.1	(-)2.8	16.6
S.E.M.[b]		5.9	4.3	7.4	4.7	7.5	6.0
Source of Variation							
Calcium (Ca)		0.07	NS	NS	0.02	NS	0.09
Phosphorus (P)		NS	NS	0.06	NS	0.07	0.0001
Ca X P		NS	NS	0.08	NS	0.03	0.02

[a]Ages at which excreta were collected.

[b]Standard error of the means.

Table 3. Experiment One: Main effects of dietary calcium and phosphorus on % phytate phosphorus retention by laying hens

Age of hens at measurement	% Phytate Phosphorus Retention					
	Calcium, %			Phosphorus Feedng Program, %		
	3.0	3.5	4.0	.64 continuous	.64-.54-.44	.56-.49-.39
Week 34[a]	52.8	40.7	46.3	50.0	48.5	41.9
Week 50[b]	8.0	11.2	8.0	12.6	-.2	14.5
Week 72[c]	22.5	16.0	11.0	11.4	25.0	13.2

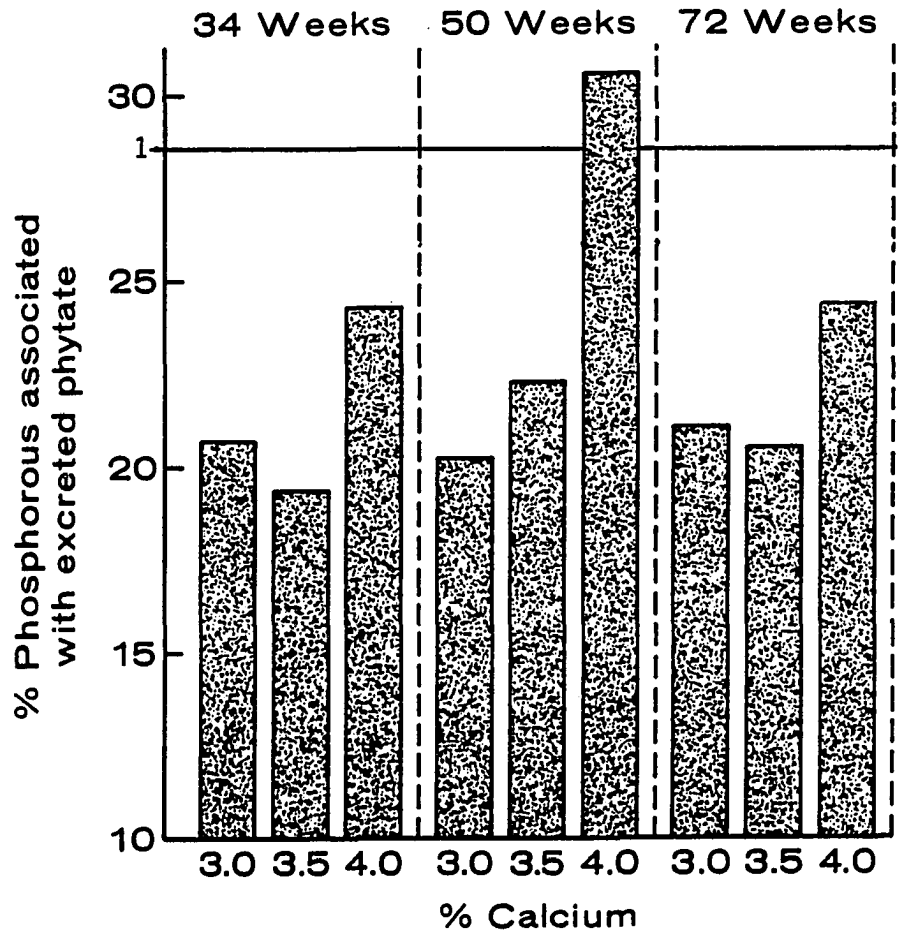
[a]Significant Ca main effect at $p<0.07$.

[b]Significant P main effect at $p<0.06$.

[c]Significant P main effect at $p<0.07$.

time of measurement to another. For example, measurements at 50 weeks were made during high temperature of midsummer whereas those at 72 weeks were made under the cooler conditions of winter.

Treatment averages showing the % of P associated with excreted phytate at each age interval are presented in Table 2, and the main effects of dietary Ca on this measurement are illustrated in Figure 2. No dietary effects on % P associated with phytate P were detected at 34 weeks of age; however, a slight increase was evident in hens fed 4.0% Ca (Figure 2). A significant ($p<0.02$) Ca main effect on % P associated with excreted phytate was observed at 50 weeks of age. At this time, hens fed 3.0, 3.5, and 4.0% Ca excreted phytate that contained 20.1, 22.6, and 31.1% P. A similar trend was observed at 72 weeks of age, when % P associated with phytate again increased as dietary Ca increased to 4.0%. A dietary P main effect ($p<.0001$) was observed at 72 weeks of age, whereby hens fed .64, .44, and .39% P had a declining % P as phytate in excreta (i.e., 32.8, 18.4, and 16.0% P), respectively. A significant Ca X P interaction effect was detected at 72 weeks of age. Hens fed .64% P had a calculated level of 43.8% P associated with excreted phytate when dietary Ca was 4.0% (Table 2). This level of 43.8% P is too high because it is impossible for phytate to bind such a high level of P. Analytical error in the determination of phytate P and phytate must have contributed to the excessive value of 43.8% P associated with excreted phytate. The amount of P associated with excreted phytate decreased when relatively low dietary P levels were fed at 50 and 72 weeks of age.



[1] 28.4% phosphorus would be associated with phytate when all six carbons are phosphorylated (hexaphosphoinositol).

Figure 2. Main effects of dietary calcium on % phosphorus associated with excreted phytate

Experiment Two

Treatment means for body weight gain, feed intake, serum P, serum ^{33}P, femur ash, femur P and ^{33}P activity, are shown in Table 4. No significant treatment effects were observed on chick body weight gain or feed intake. Serum P was significantly ($p<.002$) greater in chicks fed .80% Ca (8.23 mg P/dl) than in chicks fed 1.20% Ca (6.97 mg P/dl). However, serum ^{33}P activity did not differ between the two Ca levels. Femur ash was relatively greater in chicks fed 1.20% Ca (.337 g) than in chicks fed .80% Ca (.315 g). The positive effects of Ca on bone growth were also detected in the amount of ^{33}P activity/femur, which was significantly ($p<.002$) greater in chicks fed 1.20% Ca (74,000 DPM/femur) than in chicks fed .80% Ca (69,000 DPM/femur). Percent P in the femur did not vary with dietary treatment.

Means for non-phytate P, phytate P, non-phytate ^{33}P, and phytate ^{33}P activity in excreta dry matter, and % P associated with excreted phytate, are shown in Table 5. Percent phytate P and non-phytate P in excreta dry matter did not vary with dietary treatment at either 24 or 48 hours following ^{33}P dosing. The concentration of non-phytate P in excreta was considerably greater than that of phytate P. Non-phytate ^{33}P activity was significantly ($p<.001$) greater in chicks fed the low Ca diet (.80%) than in chicks fed 1.20% Ca, at 24 hours post ^{33}P dosing. At 48 hours post ^{33}P dosing, non-phytate ^{33}P activity in excreta decreased and the Ca dietary effect was no longer significant. Phytate ^{33}P activity in the excreta was much less than that of non-phytate ^{33}P activity and was almost non-existent at 48 hours post ^{33}P dosing. Dietary Ca did not

Table 4. Experiment two: final body weight, feed intake, tissue phosphorus and tissue ^{33}P activity

Measurement	% Calcium .80	1.20	SEM[a]
Body weight gain (g/2 chicks/week)	363	352	10.2
Feed Intake (g/2 chicks/day)	83.2	82.1	1.83
Serum P (mg/dl)	8.23*	6.97	.226
Serum ^{33}P (DPM/.1ml)	1170	1380	110
Femur Ash (g/femur)	.315	.337	.015
Femur ^{33}P (DPM/femur)	69900*	74000	4490
Femur P (%)	18.61	18.51	.488

[a]Standard Error of the Mean.

*Significant Students T-test (.80 vs. 1.20% Ca) at $p<.002$.

Table 5. Experiment two: phytate P and non-phytate P, phytate ^{33}P and non-phytate ^{33}P activity in excreta dry matter, and % P associated with excreted phytate

Measurement		% Calcium .80	1.20	SEM
Non-phytate P (%)	Day1[a]	1.99	1.80	.09
	Day2[b]	1.95	1.91	.07
Phytate P (%)	Day1	.25	.31	.03
	Day2	.32	.31	.03
Non-phytate ^{33}P (DPM/g excreta)	Day1	10770*	3480	325
	Day2	3770	720	190
Phytate ^{33}P (DPM/g excreta)	Day1	200	220	25
	Day2	23	30	4
% P associated with excreted phytate	Day1	5.74	6.37	.78
	Day2	7.58	7.11	.86

[a]24 hour collection time post 33P dosing

b48 hour collection time post 33P dosing

*Significant Students T-Test (.80 vs. 1.20% Ca) at $p<.001$.

affect phytate ^{33}P activity or % P associated with excreted phytate. The % P associated with excreted phytate ranged from 5.74 to 7.58%, which is considerably less than the 28.4% P that would be expected if the phytate were hexa-phosphoinositol. These results indicate that partial hydrolysis of phytate P occurred during digestion in these young chicks. Percent P associated with dietary phytate was 11.13% and 10.07% for the .80 and 1.20% Ca diets, respectively, which implies that some dietary phytate moieties have less that six phosphate groups bound to them. Error in the laboratory analysis of phytate P may have contributed to the observation of low dietary phytate P values which is considerably less than the 19% P associated with the dietary phytate source. A calculation of dividing 5.74 to 7.58% P associated with excreted phytate by the analytical average value of 11% P associated with total dietary phytate gives an estimate of approximately 31 to 48% phytate P hdyrolysis in the chicks of this experiment. The magnitude of this hydrolysis was not affected by dietary Ca level.

DISCUSSION

With no evident dietary effects on production traits in experiment one, it can be assumed that the hens were not overtly deficient in dietary Ca or P. Nevertheless, significant dietary effects were observed on phytate P retention and % P associated with excreted phytate. Average % phytate P retention was quite high at 34 weeks of age, averaging 46.7%, decreasing thereafter to 9.1 and 16.5% at 50 and 72 weeks of age, respectively. These data indicate that appreciable hydrolysis of phytate occurred in the laying hen during early egg production, but declined to low levels later on. Previously, Nelson (1967) reported changes in phytate P hydrolysis as chickens aged up to maturity. In this instance, however, the proportion of dietary phytate hydrolyzed increased with age, whereas, in the first experiment reported herein, the opposite occurred as hens progressed through the egg laying period. In experiment two, low levels of % P associated with phytate in excreta indicate extensive hydrolysis of phytate P in the young chicks. Percent phytate P hydrolysis in these young chicks ranged from 31 to 48 % which is comparable to the range of values reported by Ballam et al. (1985) i.e., 42 to 56% phytate P hydrolysis in chicks fed .09% dietary Ca. However, Ballam et al. (1985) also reported a significant decrease in phytate P hydrolysis from 49.5 to 7.1% as dietary Ca increased from 0.09 to 1.00%, respectively. No significant dietary Ca effects on phytate P hydrolysis were observed in the chick experiment reported herein.

The season during which experiment one excreta collections were made may also have affected phytate P retention. Collections made at 34, 50,

and 72 weeks of age were during the months of May, August, and February, respectively. The large decrease in phytate P retention at 50 weeks of age could have been influenced by heat stress. Charles et al. (1978) found that the laying hen had a higher % dietary P requirement during hot ambient temperatures but they attributed this to a decrease in total feed intake. The dietary Ca effect on % phytate P retention was not as pronounced at 50 weeks as compared with 34 and 72 weeks in experiment one and it could be inferred that the high temperature at 50 weeks modified dietary Ca's effect on phytate P retention.

Significant effects of dietary Ca and (or) P on phytate P retention were found during all three age intervals. Increasing dietary Ca from 3.0 to 3.5 and 4.0% decreased % phytate P retention. This adverse effect of increasing dietary Ca on phytate P utilization has been observed previously by Nelson (1976), and Ballam et al. (1984 and 1985). Effects of dietary P on phytate P retention were inconsistent from 50 to 72 weeks of age. The inconsistency of dietary effects on % phytate P retention could be the result of a number of factors. These factors include wide variation among hens fed the same diet and (or) variation in the analytical procedures used to measure phytate P and phytate. Large standard deviations were observed for treatment means of % phytate P retention at 50 and 72 weeks of age. Error in the analytical procedures used to determine phytate and phytate P are also possible. As described in the material and methods section, phytate P was precipitated from the TCA extract by the addition of $FeCl_3$. It is plausible that the $FeCl_3$ may be causing the precipitation of more P than just phytate P, which could

alter the accuracy of the phytate P measurement. It is therefore difficult to make solid conclusions from these data; instead, generalizations must be made about dietary trends. In general, the data reported herein did not show a consistent dietary P effect on phytate P retention such as Ballam et al. (1981 and 1984) found. They found an increase in phytate P retention when low levels of non-phytate P were fed to poultry.

In experiment two, P excretion, expressed as % phytate P or % non-phytate P in excreta dry matter, was not affected by dietary Ca. However, the excretion of ^{33}P was greater in chicks fed the low Ca diet (.80%). Increased femur ^{33}P activity and less ^{33}P activity in excreta of chicks fed 1.20% Ca indicated increased P utilization in chicks fed the higher Ca diet. This supports the research reported by Scheideler and Sell (1986), in which higher levels of dietary Ca fed to laying hens increased total P (non-phytate P + phytate P) retention. Scheideler and Sell (1986) measured total P retention at 34, 42, 50, 62 and 72 weeks of age and reported average total P retentions of 104, 148, and 178 mg/day in hens fed 3.0, 3.5, and 4.0% Ca, respectively. Dietary Ca had the opposite effect on phytate P retention in laying hens, i.e., phytate P retention decreased as Ca increased from 3.0 to 3.5 and 4.0%. It appears that dietary Ca has a different effect on non-phytate P utilization than on the utilization of phytate P by poultry.

Percentage P associated with phytate indicates the amount of P bound to the phytate molecule. If the hydroxyl groups on all six carbons of the inositol ring are phosphorylated (hexa-phosphoinositol), phytate

should contain 28.4% P. As hydrolysis of phytate occurs, the % of P associated with the molecule would decrease. The phosphate groups of phytate also may bind cations such as Ca. A Ca-phytate chelate often forms in the gastrointestinal tract (Wise, 1983), and because this complex is relatively insoluble, hydrolysis of phytate can be impaired. The data of experiment one indicate that increasing Ca from 3.0 to 4.0% of the diet may have increased the amount of P associated with phytate of excreta. At 50 and 72 weeks of age, hens fed 4.0% Ca and 0.64% P had greater than 28.4% P associated with the excreted phytate. Greater than 28.4% P associated with phytate could be the result of a Ca-phytate chelate binding non-phytate P. This is possible considering that Ca has two ligands with strong binding affinity for phosphate groups; thus, each Ca ion could bind two phosphate groups concurrently. An illustration of a possible Ca-phytate chelate binding a non-phytate phosphate group is shown in Figure 3. Actual instances of % P associated with excreted phytate exceeding 28.4% occurred only four times during the three measurement periods. However, binding of non-phytate P by the phytate molecule could occur whenever a cation (Ca+) or phytate ligand is available for binding. Data of experiment two support the theory of phytate binding of non-phytate P. ^{33}P activity was found associated with excreted phytate in the second experiment. The ^{33}P activity could have bound with phytate at available cation ligands as depicted in Figure 3, or ^{33}P could have been exchanged with phytate P on the phytate moiety itself. Gillis et al. (1957) reported a flux of ^{32}P between phytate P and tissue P indicating an exchange of phytate P for non-phytate P. No

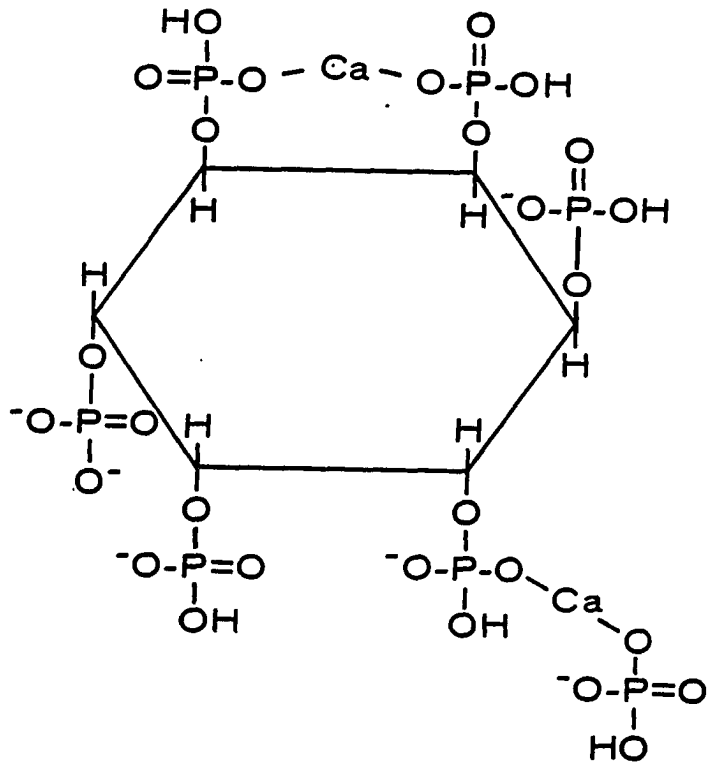


Figure 3. Phytate-calcium chelate with non-phytate phosphorus

dietary Ca effect was observed on the amount of ^{33}P associated with phytate excreted by chicks of experiment two.

The results of these experiments give evidence that young chicks and laying hens are capable of utilizing some phytate P; however, the extent of phytate P utilization by laying hens is modified considerably by dietary Ca and decreases as the hen ages. Data from experiment two showed no dietary Ca effect on phytate P utilization in one-week-old chicks, but bone ^{33}P deposition was greater and excreted ^{33}P activity was less in chicks fed 1.20% Ca indicating increased overall P utilization in the chicks fed the higher level of dietary Ca.

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SECTION III. INFLUENCE OF DIETARY CALCIUM ON PHOSPHORUS ABSORPTION, EXCRETION AND ^{33}P DISTRIBUTION IN POULTRY

ABSTRACT

Two experiments were conducted utilizing isotope-dilution and comparative balance techniques to estimate urinary and fecal phosphorus (P) excretions by laying hens fed varying levels of dietary calcium (Ca). In experiment one, hens were injected with 50 uCi ^{33}P and tissue samples (plasma, liver, and kidney) were taken from two hens at 14 and 22 hours post-^{33}P dosing and six hens at 34, 42, and 58 hours post-^{33}P dosing. Relative specific activities (RSA) were calculated as the ratio of tissue specific activity (DPM/mg P) to plasma specific activity (DPM/mg P). Kidney and liver tissue RSAs were similar (.77 and .79) during the 34 and 42 hour collection times. At 58 hours, ^{33}P specific activity accumulated in the liver and kidney tissues to a RSA greater than 1.0. In experiment two, each of two levels of dietary Ca (3.46 and 4.2%) was fed to eight hens for 30 days, during which time, daily egg production and feed intake were recorded. After 30 days, hens were injected with 50 uCi ^{33}P two hours post-oviposition. Forty-eight hours after ^{33}P dosing and one hour post-oviposition, plasma, liver, kidney, femur bone, whole egg, ileum, ileal digesta and excreta samples were collected from each hen. All tissues were analyzed for P and ^{33}P activity. P retention (mg/hen daily) and P balance, including egg P, (mg/hen daily) were determined for each hen. Results showed a favorable effect of increasing dietary Ca (3.46 vs. 4.2%) on egg production (91.9 vs. 96.6%), femur bone ash (55.98 vs. 57.75%), P retention (17.9 vs. 135.0 mg/hen daily), and P balance (-74.0 vs. 38.4 mg/hen daily). Hens fed the 3.46% Ca diet excreted more P than did those fed 4.2% Ca. Since ileal P was not affected by dietary Ca, but

excreta P was increased by low Ca, the source of increased P in excreta of hens on the low Ca diet was urinary P likely released during medullary bone mobilization for egg shell formation. Endogenous P secretions were estimated to constitute less than 1% of the P in ileal digesta and in excreta samples with no dietary Ca effects observed. In summary, the results of these experiments demonstrated that laying hens need adequate Ca intake to prevent excessive bone reabsorption and urinary P excretion. Increasing dietary Ca did not affect P absorption or the secretion of endogenous P in the intestinal tract, but decreased total P excretion.

INTRODUCTION

The effects of dietary calcium (Ca) on phosphorus (P) absorption and excretion have been reported by several investigators, but the results were inconsistent. Hurwitz and Bar (1965) found that the % P absorption by hens was depressed as dietary Ca increased from 1.9 to 3.56%. This effect of Ca occurred despite a notable decrease in the excretion of endogenous P by hens fed low Ca diet. No data on absolute P retention were given. However, decreases in absolute P retention as dietary Ca increased from 3.5 to 4.5 and 5.5% in laying hen diets were reported recently by Keshavarz (1986).

In contrast with the aforementioned observations, Fox and Care (1978) reported enhanced intestinal P absorption in chicks fed diets high in calcium. Similarly, Scheideler and Sell (1986) reported positive effects of increasing dietary Ca from 3.0 to 3.5 and 4.0% on P retention in laying hens at various times during egg production.

The consistency and magnitude of these positive effects of Ca on P retention indicated a need for research designed to determine the mechanisms involved. Two primary mechanisms seem feasible; increasing dietary Ca may enhance P absorption and (or) increasing dietary Ca may reduce P excretion by laying hens. Determination of P absorption in poultry is difficult because of the combined excretion of urine and feces through the cloaca. Research done with colostomized birds would enable the separate collection of urine and feces; however, colostomy alters the natural state of the bird and may change gastro-intestinal function. An alternative approach would be to include a non-absorbable marker in the

diet, collect excreta for a prescribed period, and then obtain digesta from the terminal ileal area. The comparative-balance technique of relating the P concentration per unit of marker in the diet, ileal digesta, and excreta could be used to estimate apparent P absorption and urinary P excretion. Additional information concerning P absorption could be obtained by differentiating P in ileal digesta of dietary origin versus that originating from endogenous P secretions into the intestinal tract. The isotope-dilution procedure, as employed by Brown and McCracken (1965) could be used for this purpose. In this instance, ^{32}P was injected intramuscularly into chickens and the proportion of radioisotope observed in digesta was used to estimate endogenous P secretion.

Guenter and Sell (1973) utilized a combination of the comparative-balance and isotope-dilution techniques to study magnesium absorption and excretion in chickens. A combination of these techniques involving the use of chromic oxide as a non-absorbable marker and ^{33}P as the radioisotope was chosen as a means for determining the effects of dietary Ca level on P absorption, endogenous P secretion and urinary P excretion by laying hens. Two experiments were conducted.

The objective of experiment one was to measure the amount of time needed for ^{33}P, injected intramuscularly, to equilibrate among selected tissues (plasma, liver and kidney) of hens. The objective of experiment two was to test the effects of dietary Ca (3.46 and 4.2%) on phosphorus absorption, endogenous P secretion and urinary P excretion by the laying hen.

MATERIALS AND METHODS

Experiment One

Twenty-two Single Comb White Leghorn hens 83 weeks old were housed individually in cages (50cm x 40cm x 30cm) equipped with individual feed pans and excreta collection trays. All hens were fed a corn-soybean meal basal diet containing 2990 kcal ME/kg, 16% protein, 2.96% calcium, .37% available phosphorus and .59% total phosphorus. Lighting was provided for 14 hours daily and environmental temperature was controlled at 25^{o}C.

Hens were fed two times daily, at 0700 and 1500 hours, ad libitum. At 2400 hours on day 9 of the experiment, 40 uCi ^{33}P (1 Ci/mmole sodium monohydrogen phosphate in water (New England Nuclear Products, Boston, MA.) was injected intramuscularly into each hen. Two hens were subsequently killed at 14 and 22 hours post ^{33}P dosing, and six hens were killed at 34, 42, and 58 hours post ^{33}P dosing to obtain blood, liver and kidney samples. Collection times were coordinated to occur between 1 1/2 and 3 hours after feeding times.

Blood was centrifuged and plasma was collected. Liver and kidney samples were immediately frozen in liquid N after excision, freeze-dried and then homogenized with a mortar and pestle. Plasma inorganic P was measured by spectrophotometry by using a P AUTO/STAT Kit (Lancer Chemistries and Analytical Systems, Foster City, CA.). Liver and kidney samples were prepared for P analysis by ashing a portion of the dry tissue and dissolving the ash in dilute HCl. An aliquot of the aqueous tissue sample was analyzed for P by a colorimetric auto-analyzer procedure according to Association of Official Chemists methods (1980).

Plasma, liver and kidney ^{33}P activities were counted on a Packard Tri-Carb 300 liquid scintillation system (Packard Instrument Co. Downers Grove, Ill.). Counting efficiency for ^{33}P exceeded 90%. Aliquots of plasma and aqueous liver and kidney tissue preparations were combined with 10 ml Beckman Ready-Solve scintillation cocktail (Beckman Instruments, Inc., Fullerton, CA.) and then were counted with the Packard scintillation counter. Quench correction curves were prepared for each tissue by standard channels ratio.

Relative specific activity (RSA) was calculated as the ratio of the specific activity (DPM/mg P) of tissue (liver and kidney) to the specific activity of plasma. The RSA of each tissue was determined at each collection time (14, 22, 34, 42, and 58 hour post ^{33}P dosing) during the experiment.

Experiment Two

Sixteen 28-week-old SCWL hens were housed in individual cages (50cm x 40cm x 30cm) equipped with excreta collection trays. Eight hens were assigned randomly to each diet (3.46 or 4.2% Ca by analysis). Diet compositions are shown in Table 1. Chromic oxide and celite were included in the diets at a level of 1% each to function as non-absorbable markers. Hens were fed ad libitum in individual feed pans each morning at 0730 hours. Lighting was provided for 14 hours daily and temperature was maintained at 25^{o}C. Feed intake and egg production were recorded daily and body weight was measured at the end of the experiment.

After 30 days on the experimental diets, hens were injected intramuscularly with 50 uCi ^{33}P (1 Ci/mmole) (same form as in Experiment

Table 1. Experiment two: Composition of experimental diets

Ingredient	% Calcium 3.46	4.2
Ground Yellow Corn	65.22	57.88
Soybean Meal	23.26	24.59
Fat	.12	2.81
Limestone	7.25	10.59
Dicalcium Phosphate	1.26	1.33
Chromic Oxide	1.00	1.00
Celite	1.00	1.00
Methionine	.09	.10
Vitamin Premix[a]	.50	.50
Mineral Premix[b]	.30	.30
Calculated Analysis		
Metabolizable Energy kcal/kg	2820	2820
Protein, %	17.0	17.0
Calcium, % (calculated)	3.0	4.2
Calcium, % (analyzed)	3.46	4.2
Phosphorus, available, %	.34	.35
Phosphorus, total, %	.56	.56
Lysine, %	.89	.91
TSAA, %	.68	.68

[a]Contributed per kilogram of diet: vitamin A, 4000 I.U.; vitamin D3 1500 I.U.; vitamin B12, 5 ug; riboflavin, 3.5 mg; pantothenic acid, 6.0 mg; niacin, 22 mg; choline, 100 mg.

[b]Contributed per kilogram of diet: manganese, 20 mg; sodium chloride, 2.95 g; iodine, .23 mg.

one) at two hours post-ovipositon. All eggs produced for two days following ^{33}P dosing were gathered and saved for further analysis. Two days after ^{33}P dosing, (a minimum of 48 hours), blood was obtained by heart puncture at one hour post-oviposition. The blood was immediately centrifuged to collect plasma. The hens were killed by euthanasia and liver, kidney, femur bone, ileal digesta, and ileum samples were collected from each hen. All samples, with the exception of the femur bone, were frozen in liquid N, freeze-dried and homogenized with a mortar and pestle. Femur bone samples were cleaned, fat extracted and ashed overnight at 600^{o}C in a muffle furnace. Excreta samples were collected from each hen from the time of the morning feeding (0730 hours) on day 2 post-^{33}P dosing until the time of kill at one hour post-oviposition. Excreta samples were freeze-dried and homogenized by mortar and pestle. Diet samples were collected and were finely ground in a blender.

Whole eggs were weighed and then broken to separate liquid contents and shell. Shell thickness was determined by averaging micrometer readings taken at three points around the equator of each shell. The remaining egg was freeze-dried and homogenized in a blender.

Plasma inorganic P was measured spectrophotometrically. Liver, kidney, ileum, femur bone, egg, ileal digesta, excreta, and diet samples were prepared for P analysis by the same procedure used in experiment one for tissue P analysis. Plasma ^{33}P and tissue ^{33}P activities were counted by the same methods used for experiment one. RSA of each tissue also was calculated.

Diet, ileal digesta and excreta samples were analyzed for chromic

oxide content spectrophotometrically by the procedure described by Fenton and Fenton (1979). Acid insoluble ash was measured in diet, ileal digesta and excreta by a modified procedure from the Association of Official Analytical Chemists methods (1980).

Ileal digesta, excreta and urinary P were expressed in units of mg P/mg Cr_2O_3 in the dry matter. Endogenous P in ileal digesta and excreta was calculated and subtracted from the total P value in each material to give the corrected values reported herein. Phosphorus retention and P balance were also calculated for each dietary treatment. Calculations of endogenous P in ileal digesta and excreta, urinary P, P retention and P balance were made as follows:

Endogenous P in ileal digesta = RSA of ileal digesta x total P/Cr_2O_3 ratio of ileal digesta.

Endogenous P in excreta = RSA of excreta x total P/Cr_2O_3 ratio of excreta.

Urinary P (mg P/mg Cr_2O_3) = Excreta P (mg P/mg Cr_2O_3) - Ileal digesta P (mg P/mg Cr_2O_3).

P Retention (mg/hen daily) = mg P intake/hen daily - mg P excreted/hen daily.

P Balance (mg/hen daily) = P retention (mg/hen daily) - egg P loss (mg/hen daily).

Means were compared by using Students T-test and data were analyzed by using the Statistical Analysis System (SAS) developed by the SAS Institute, Cary, NC. (Barr et al., 1982).

RESULTS AND DISCUSSION

Experiment One

The specific activities of ^{33}P (SA) obtained for plasma, liver and kidney were used to calculate the relative specific activities (RSA) of ^{33}P where liver or kidney SA's/plasma SA equalled RSA. The resulting RSAs for liver and kidney for each sampling time are shown in Table 2. Variation among individual hens within a sampling time was relatively large and, consequently, statistically significant differences between tissues or sampling times were rare. Only in the instance of kidney measurements at 14 and 22 hours were the RSAs significantly different from the unity value of plasma. The high variation in specific activity may have been a result of the following factors; unequal rate of egg production among the replicates, old age of the hens (83 weeks) and in production for over 1 year, and the factor of tissue samples not being collected at a uniform time relative to oviposition.

Numerically, there seemed to be a rapid influx of ^{33}P into the liver by 14 hours post-injection of the radioisotope followed by a relatively stable set of RSAs at 22, 34, and 42 hours post-injection times. The RSA of liver ^{33}P again reached a high level after 58 hours. Kidney RSAs remained fairly low through 22 hours post-injection and then reached levels equivalent to those of liver at 34, 42, and 58 hours. These data suggest that ^{33}P moved into the liver more rapidly than the kidney but that by 34 hours post-injection, ^{33}P stabilized at a similar level in both tissues relative to the plasma.

Table 2. Experiment one: relative specific activities (RSA) of soft tissues as related to time after ^{33}P administration

Sample	Hours after ^{33}P administration				
	14	22	34	42	58
Plasma	1.0 785900+707200[a]	1.0 1474600+1173300	1.0 706800+336600	1.0 412500+168800	1.0 227600+100600
Liver	1.46+1.41[b]	.64+.41	.77+.19	.77+.20	1.47+.26
Kidney	.36[c]	.38+.21	.74+.27	.84+.31	1.34+.36

[a]DPM/g P in plasma.

[b]Means + standard deviation.

[c]One hen observation.

Despite the large variation among individual hens which obscured statistically significant differences that may have existed, the numerical values suggest that true equilibration of ^{33}P in liver and kidney with that in plasma was not achieved at any sampling time.

An explanation of why the soft tissues specific activities were not in equilibrium with plasma specific activities could be that not all of the P present in the liver and kidneys was readily exchangeable with plasma P, as labeled by the tracer ^{33}P. Thus, to expect an RSA of 1.0 between soft tissues and plasma may be unrealistic; rather, a stable RSA over a period of time, such as occurred in this experiment between 34 and 42 hours post ^{33}P dosing, could be the best indicator of a "short-lived" equilibrium between exchangeable P in soft tissues and plasma. Shirley, et al. (1954) injected ^{32}P into laying hens and measured the stabilization of ^{32}P among soft tissues and bones. They reported that ^{32}P stabilized in the liver and kidney only 3 hours after dosing, while stabilization in the bones took 98 hours. Also, Shirley et al. (1954) reported that 26% of the ^{32}P dose was excreted in the first 3 days and thereafter, 2.2% of the dose was excreted per day. Smith et al. (1954) conducted an experiment measuring the appearance of injected ^{32}P in different components of the egg and through extrapolation of the data came to the conclusion that ^{32}P distributes among two P pools in the laying hen. The first pool contained approximately 18% of the exchangeable P with a turnover rate of 1.4 days, whereas the second pool contained approximately the remaining 82% P and had a turnover rate of 14 days. Drawing a correlation between Smith et al.'s (1954) observations

and the results of experiment one reported herein, it would seem that during the time frame in which tissues were taken for ^{33}P activity measurements, the majority of the equilibrated activity would have been in the first pool (turnover rate of 1.4 days) and only a small fraction of the ^{33}P activity would have equilibrated in the larger pool (turnover rate of 14 days) at 58 hours post ^{33}P dosing. Thus the RSAs of soft tissues determined in this experiment were probably most indicative of the smaller P pool with the fast turnover rate (1.4 days). The P pool with the slow turnover rate (14 days) was probably indicative of the P pool in bones. The bone P pools would be very slow to equilibrate with plasma ^{33}P during the course of this experiment.

The indication of a stabilization of ^{33}P during the period 34 and 42 hours after ^{33}P administration in experiment one was used as a basis for determining the time at which tissue collections were made in the second experiment reported herein.

Experiment Two

Effects of dietary Ca on body weight, feed intake, egg production, egg weight, shell thickness, egg P, femur ash, femur P and plasma P are shown in Table 3. Significant dietary effects were observed only on egg production ($p<.08$) and % femur ash ($p<.06$). Hens fed 3.46 and 4.2% Ca laid eggs at a rate of 91.9 and 96.6%, respectively. Favorable effects of increasing dietary Ca on egg production were also reported by Scheideler and Sell (1986) where increasing dietary Ca from 3.0 to 3.5 and 4.0% increased egg production in 52 to 72 week-old-hens. Percent femur ash was 55.98 and 57.71% for hens fed 3.46 and 4.2% Ca,

Table 3. Experiment two: Effect of dietary calcium on body weight, feed intake, egg production, egg weight, shell thickness, egg phosphorus, femur ash, femur phosphorus and plasma phosphorus in laying hens

Measurement	% Calcium 3.46	4.2
Body weight, g/hen[a]	1367+43.2[b]	1367+57.2
Feed Intake, g/day/hen[c]	84.0+3.7	84.9+1.9
Egg Production, %[c]	91.9+5.4*	96.6+4.3
Egg Weight, g[d]	50.7+4.2	49.5+3.1
Shell Thickness, mm	.303+.046	.311+.016
Egg Phosphorus, mg/egg	100.6+28.3	105.7+27.3
Femur Ash, %	55.98+1.59**	57.75+1.27
Femur Phosphorus, mg/g bone ash	244.8+18.8	235.3+ 6.5
Plasma Phosphorus mg/dl	4.65+.75	5.20+1.05

[a]Body weight measured at end of 32-day experiment.

[b]Mean + Standard Deviation.

[c]Feed intake and egg production measured daily during 32-day experiment.

[d]Egg weight and remaining measurements made during the final 2 days of the trial after ^{33}P dosing.

*Significant T-Test (3.46 vs. 4.2% Ca) at $p<.08$.

**Significant T-Test (3.46 vs. 4.2% Ca) at $p<.06$.

respectively. Decreased egg production and reduced femur bone ash in hens fed the lower Ca diet indicate that Ca intake was insufficient to sustain both high egg production and bone mineralization in young pullets. The National Research Council (NRC) (1984) recommends feeding laying hens 3.40% Ca, or a total intake of 3.75 g Ca/hen daily. Data from the experiment reported herein show that Ca intakes were 2.91 and 3.57 g/hen daily for hens fed 3.46 and 4.2% dietary Ca, respectively. The relatively low Ca intake was a result of low total feed intake in these hens (84.0 to 84.9 g/day), which is considerably less than the value assumed by NRC (1984) (110 mg/hen daily). Nevertheless, the hens consuming 3.57 g/hen daily maintained a high rate of egg production (96.6%).

RSAs for liver, kidney, ileum, femur bone, whole egg, ileal digesta, and excreta are shown in Table 4. Tissues were collected 48 hours after ^{33}P administration and at a time corresponding to one hour post-oviposition. There were no significant effects of dietary Ca level on RSAs of tissues, eggs, digesta, or excreta. Also, dietary Ca did not alter the specific activity of ^{33}P in plasma. The RSAs of the soft tissues at 48 hours post-^{33}P administration ranged from .59 to .81, and were fairly consistent with the RSAs observed in experiment one at 34 and 42 hours after ^{33}P administration. In both experiments, soft tissue RSAs were not in total equilibrium with plasma ^{33}P. The relative specific activities of soft tissues (liver, kidney and ileum) were significantly (T-test $p<.10$) less than that of plasma, but the tissue RSAs were similar to one another. However, as discussed in experiment one, total

Table 4. Experiment two: Relative specific activities (RSA) of soft tissues, femur bone, egg, ileal digesta and excreta 48 hours after 33P dosing

Tissue	% Calcium 3.46	4.20
Plasma	1.00 (417,300+115,300[a])[b]	1.00 (478,600+114,000)
Liver	.59+.15	.60+.08
Kidney	.64+.20	.58+.21
Ileum	.81+.35	.64+.10
Bone	.020+.011	.018+.007
Egg	.047+.040	.031+.007
Ileal Digesta	.0026+.0010	.0022+.0011
Excreta	.0039+.0006	.0041+.0023

[a]Mean + Standard Deviation.

[b]DPM/g P in plasma.

equilibrium of exchangeable P between soft tissues and plasma, as measured by the tracer ^{33}P, may not be plausible because of unequal exchange of ^{33}P with the P pools present in the tissues. Nonetheless, the soft tissue specific activities were relatively stable in relation to each other.

Femur bone and whole egg RSAs were considerably less than those of soft tissues. Femur bone RSAs are representative of the ^{33}P used for new bone mineralization as well as the P exchanged with bone P pools. Low specific activity in the bones at 48 hours post-^{33}P dosing was likely due to the relatively slow exchange of P pools in ossified bone material and the slow mineralization process. Shirley et al. (1954) reported a stabilization of ^{32}P in bones 98 hours after injection. A low whole egg RSA is not surprising considering that the majority of egg P is in the yolk and that the yolk takes 9 days to mature before ovulation. Therefore, the small amount of ^{33}P activity that was present was probably associated with the albumen which takes less than 24 hours to form. These results are supported by the research of O'Neil (1948) who reported ^{32}P activity in the egg shell 24 hours after dosing, in the egg white 48 to 72 hours after dosing, and in the egg yolk 144 hours after dosing.

The RSAs of ileal digesta were very low indicating that little ^{33}P was secreted endogenously into the intestinal tract. Also, the ^{33}P of the excreta was only slightly greater than that of ileal digesta. The latter data suggest that very little plasma ^{33}P was being excreted in the urine, and the combined losses of endogenous ^{33}P via feces and the ^{33}P excreted via urine were quite small in comparison to the levels of ^{33}P in

plasma. Klinefelter et al. (1984) reported that not all of the P secreted into urine comes directly from plasma P in the domestic fowl. They suggested that P pools within the kidney may also serve as a source of P secretions. Wideman and Braun (1981) infused ^{32}P into the renal-portal system of the fowl and found that ^{32}P serves as an accurate tracer for P entering the urine by glomerular filtration or for P undergoing tubular reabsorption, but net ^{32}P secretion was difficult to demonstrate. The secreted P was derived from a pool that does not readily equilibrate with ^{32}P or plasma P. The results of Klinefelter et al. (1984) and Wideman and Braun (1981) give support to the results of experiment two reported herein, in which excreted specific activity was relatively low in relation to plasma specific activity, indicating little transfer of plasma ^{33}P into the urine of these hens.

Treatment means for ileal digesta P, excreta P, urinary P, P retention and P balance are shown in Table 5. Laboratory analysis of chromic oxide and acid insoluble ash resulted in chromic oxide having less analytical variation than acid insoluble ash. Thus, chromic oxide ratios were used to obtain the values given in Table 5. Endogenous P in ileal digesta and excreta (mg P/mg Cr_2O_3) was calculated by the equation: ileal digesta or excreta RSA X total mg P/mg Cr_2O_3. Ileal digesta P and excreta P shown in Table 5 were corrected for endogenous P contributions by subtraction of endogenous P from the total mg P/mg Cr_2O_3 present in ileal digesta and excreta. Endogenous P contributions to ileal digesta P and excreta P were low in this experiment, constituting less than 1% of the total P present in these samples. This value was less than that

Table 5. Experiment two: effect of dietary calcium on ileal digesta phosphorus, excreta phosphorus, urinary phosphorus, phosphorus retention, and phosphorus balance

Measurement	% Calcium 3.46	4.2
Ileal Digesta Phosphorus mg P/mg Cr_2O_3 in Dry Matter	.58+.22[a]	.64+.25
Excreta Phosphorus mg P/mg Cr_2O_3 in Dry Matter	.86+.11*	.69+.14
Urinary Phosphorus[b] mg P/mg Cr_2O_3 Dry Matter	.27+.20**	.048+.21
Phosphorus Retention[c] mg/hen/day	17.9+8.5**	135.0+1.35
Phosphorus Balance[d] mg/hen/day	-74.0+32.7***	38.4+47.7

[a]Mean + Standard Deviation .

[b]Urinary Phosphorus calculated as Excreta Phosphorus - Ileal Digesta Phosphorus.

[c]Phosphorus Retention, mg/hen daily = P consumed, (mg/hen daily) - P excreted, mg/hen daily.

[d]Phosphorus Balance, mg/hen daily = Phosphorus retention (mg/hen daily - Egg phosphorus (mg/hen daily).

*Significant T-Test (3.46 vs. 4.2% Ca) at $p<.03$.

**Significant T-Test (3.46 vs. 4.2% Ca) at $p<.06$.

***Significant T-Test (3.46 vs. 4.2% Ca) at $p<.08$.

reported by Brown and McCracken (1965). These researchers estimated that approximately 9% of the total fecal P output was of endogenous origin. However, on a scale of 0 to 100%, there may not be that much difference between 1 and 9% endogenous P excreted by laying hens. An explanation of why the estimates of endogenous P in the experiment reported herein were low could be that the ^{33}P had not dispersed evenly among soft tissue including the intestine, at the time collections were made and (or) that endogenous secretions of ^{33}P into the gut were readily reabsorbed by the hens of the current study. Wilkinson (1976) emphasized that, in humans, endogenous P loss was difficult to measure because of a possible preferential reabsorption of labeled digestive juice P as well as reabsorption of P of exfoliated intestinal cells. Also, it seems possible that age, reproductive activity and body P status may affect the magnitude of endogenous P secretion in hens.

Ileal digesta P (mg P/mg Cr_2O_3) was not influenced by dietary Ca level, indicating that Ca was not affecting intestinal absorption of P. However, excreta P (mg P/mg Cr_2O_3) was significantly ($p<.03$) greater in hens fed 3.46% Ca (.86 mg P/mg Cr_2O_3) than in hens fed 4.2% Ca (.69 mg P/mg Cr_2O_3). The level of P/mg Cr_2O_3 was greater in excreta than in the ileal digesta regardless of dietary level of Ca. The increase in P in excreta was due to urinary P excretions joining the unabsorbed P of the lower gut. Urinary P excretion was estimated as being equal to the difference between ileal digesta P and excreta P (Table 5). Urinary P excretion was significantly ($p<.06$) greater for hens fed 3.46% Ca (.27 mg P/mg Cr_2O_3) than for hens fed 4.2% Ca (.048 mg P/mg Cr_2O_3). The extra

urinary P of the hens fed low Ca probably came from P mobilized during medullary bone reabsorption. Hens on the low Ca diet (3.46%) were most likely mobilizing medullary bone Ca stores for shell formation at a rate greater than that of hens fed 4.2% Ca. During bone mobilization, P would be mobilized as part of the hydroxyapatite crystal. The Ca of mobilized bone salt would be used for shell formation, but most of the P would be excreted into the urine (Taylor and Kirkley, 1967). Hurwitz and Griminger (1961) also reported increased urinary P among laying hens during bone mobilization for shell formation.

Phosphorus retention was significantly greater ($p<.06$) in hens fed the high Ca diet. Hens retained 17.9 and 135.0 mg P/hen daily when fed 3.46 and 4.2% Ca, respectively. These data support the results reported by Scheideler and Sell (1986) in which P retention increased in laying hens as dietary Ca increased from 3.0 to 3.5 and 4.0%. P balance (includes P deposited in eggs) was negative (-74.0 mg /hen daily) for hens fed 3.46% Ca, but was positive (38.4 mg/hen daily) for hens fed 4.2% Ca. A negative P balance in laying hens fed low levels of dietary P and insufficient dietary Ca was previously reported by Hurwitz and Griminger (1962). Summers et al. (1976) reported negative P retention by hens fed diets low in both Ca (1.50%) and P (.42%). Negative P balance in hens fed 3.46% Ca in the experiment reported herein was likely the result of increased bone reabsorption to satisfy Ca needs together with limited absorption of dietary P.

In summary, the use of isotope-dilution and comparative-balance techniques in this paper demonstrated that dietary Ca level affected P

excretion, P retention, and P balance. Urinary and excreta P were greater in hens fed 3.46% Ca as compared with P excretion by hens fed 4.2% Ca. Consequently, P retention and P balance were greater in hens fed 4.2% Ca. Dietary Ca had no effect on ileal digesta P levels, indicating that P absorption was not altered measureably by feeding either 3.46 or 4.2% Ca. Estimates of endogenous P contributions to ileal digesta P and excreta P were less than 1% of the total P in these materials, irrespective of dietary Ca level.

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GENERAL SUMMARY

A series of experiments was conducted to achieve the following objectives: 1. to assess the utilization of total phosphorus (P) and phytate P in the laying hen as influenced by dietary calcium (Ca), phase feeding P programs, strain of hen and age of hen; 2. to test the effects of dietary Ca on ^{33}P distribution and phytate P utilization in young broiler chicks, and; 3. to estimate the effects of dietary Ca on P absorption and excretion in the laying hen.

The results of experiment one demonstrated the adequacy of phase feeding P programs, (.64, .54, and .44 or .56, .49, and .39% P fed when hens were 24 to 36, 36 to 52, and 52 to 72 weeks of age, respectively) to support egg production characteristics throughout a 336-day trial. Strain effects were observed in egg weights, shell thickness, feed intake, P and Ca intake, femur ash, serum P and Ca, and P retention at 34 weeks of age. Femur ash and serum P were greater in hens fed the higher levels of dietary P. Dietary Ca had no effect on egg production characteristics in this experiment. Average total P retention based on weighted means was 104, 148, and 178 mg/hen daily when hens were fed 3.0, 3.5, and 4.0% Ca, respectively. The favorable affect of increasing Ca on total P retention was most noticeable from 50 to 72 weeks of age. At 42 weeks of age, ambient temperature was unusually hot and feed intake decreased markedly. This increase in temperature modified the effect of dietary Ca on total P retention, whereby P retention decreased as dietary Ca increased. P feeding programs had an inconsistent effect on P

retention during this experiment. An interaction effect of Ca and P on P retention was present at 62 and 72 weeks of age, with hens fed .64% P and 4.0% Ca retaining less P as compared to hens being phase fed P together with 4.0% Ca. The favorable effects of high Ca on P retention at these times, however was greatest when .49 and .39% P was fed. P retention tended to decline with increasing age of the hens. Overall, hens were in a marginal to negative P balance at 42, 62, and 72 weeks of age when P deposited in eggs and P excreted were compared with P consumed.

Phytate P retention during experiment one was relatively high at 34 weeks of age, averaging 46.6%, and then declined to 9.1 and 16.5% at 50 and 72 weeks of age, respectively. As observed with the P retention data, P feeding program had an inconsistent effect on % phytate P retention. Dietary Ca had an adverse effect on % phytate P retention at 50 and 72 weeks of age. Also, as Ca increased from 3.0 or 3.5 to 4.0%, the % P associated with phytate in excreta increased. In some instances, hens fed 4.0% Ca excreted phytate that contained greater than 30% P.

Results of experiment two (Section II), indicated increased P retention and greater bone deposition in chicks fed 1.2 than those fed .8% Ca. Serum P and excreta ^{33}P activity were greater in chicks fed .8% Ca while femur ^{33}P activity was greater in chicks fed 1.2% Ca. The % P associated with phytate in excreta ranged from 5.6 to 7.0% and phytate P utilization ranged from 31 to 48%. These estimates of phytate P hydrolysis were greater than those observed with laying hens in experiment one. There was also evidence in this experiment of a phosphate flux between the inorganic ^{33}P pool and the phytate P pool, or

the binding of ^{33}P to "free" hydroxyl groups of phytate molecules.

The final experiments (Section III) tested the effects of increasing dietary Ca on P excretion in laying hens. The radioisotope ^{33}P was injected intramuscularly and approximately 34 to 42 hours were required for the isotope to stabilize in liver and kidney tissues. However, the RSAs of these soft tissues as compared with plasma, were not equal to 1.0, indicating that ^{33}P was not fully equilibrated with the P in these tissues.

Favorable effects of increasing dietary Ca (3.46 vs. 4.2%) were observed on egg production (91.9 vs. 96.6%), femur bone ash (55.98 vs. 57.75%), P retention (17.9 vs. 135.0 mg/hen daily), and P balance (-74.0 vs. 38.4 mg/hen daily). Hens fed 3.46% Ca had increased P in excreta and the source of this P was most likely urinary P excretion. Endogenous P secretions were estimated at less than 1% of the P in ileal digesta and excreta, with no dietary Ca effects observed.

The results of these experiments showed that dietary Ca had a favorable effect on total P retention and P balance by preventing excessive bone resorption and decreasing urinary P excretion in the laying hen. However, the effects of dietary Ca on phytate P retention were the opposite of those seen on total P utilization. Increasing dietary Ca inhibited phytate P utilization and tended to increase the % P associated with phytate in excreta. Dietary P had no consistent effects on either total P or phytate P retention. The results of the long-term laying hen experiment showed that the ability to utilize P, especially P from phytate, decreases with increasing age.

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APPENDIX A: PREPARATION OF TISSUE SAMPLES FOR PHOSPHORUS, PHYTATE[1] AND PHYTATE PHOSPHORUS ANALYSIS

Excreta and digesta samples were collected in petri dishes, frozen and freeze-dried in a freeze-dryer (Virtis Co. Inc., Gardiner, N.Y.) for 48 to 72 hours. Excreta and digesta samples were then finely ground in a blender or mortar and pestle, depending on sample size, to pass through a 20-mesh sieve. Representative feed samples were taken from each diet and ground to pass through a 20-mesh screen.

Bone samples were autoclaved for 3 to 4 hours and then cleaned of all muscle and connective tissue. The clean bones were then fat-extracted by a Goldfish fat extraction apparatus, (Laboratory Construction, Co., Kansas City, MO.) to remove lipids. Bones were dried overnight at 100^{o}C and dry weight was recorded. Bones were ashed overnight in porcelain crucibles at 600^{o}C in a muffle furnace. Bone ash weight was recorded and bones were finely ground by mortar and pestle.

Whole egg samples (without eggshell) were freeze-dried for 72 hours and finely homogenized in a blender. Liver, kidney and ileum samples were frozen in liquid N immediately following excision and freeze-dried for 48 to 72 hours. Tissue samples were finely ground by a mortar and pestle.

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[1]Phytate terminology is used inclusively to represent all forms of phytic acid present and measured in the samples.

APPENDIX B: COLORIMETRIC DETERMINATION OF PHOSPHORUS BY TECHNICON AUTO-ANALYZER TECHNIQUE

Two hundred and fifty milligrams of prepared kidney, liver and ileum tissue (Appendix A) were weighed into porcelain crucibles and ashed in a muffle furnace for 24 hours at 600°C. After ashing, the crucibles were cooled and 10 ml of 3N HCl were added to each crucible. The crucibles were placed on a hot plate and brought to a boil for 30 to 60 seconds. Samples were then transferred to 10 ml volumetric flasks and diluted to volume.

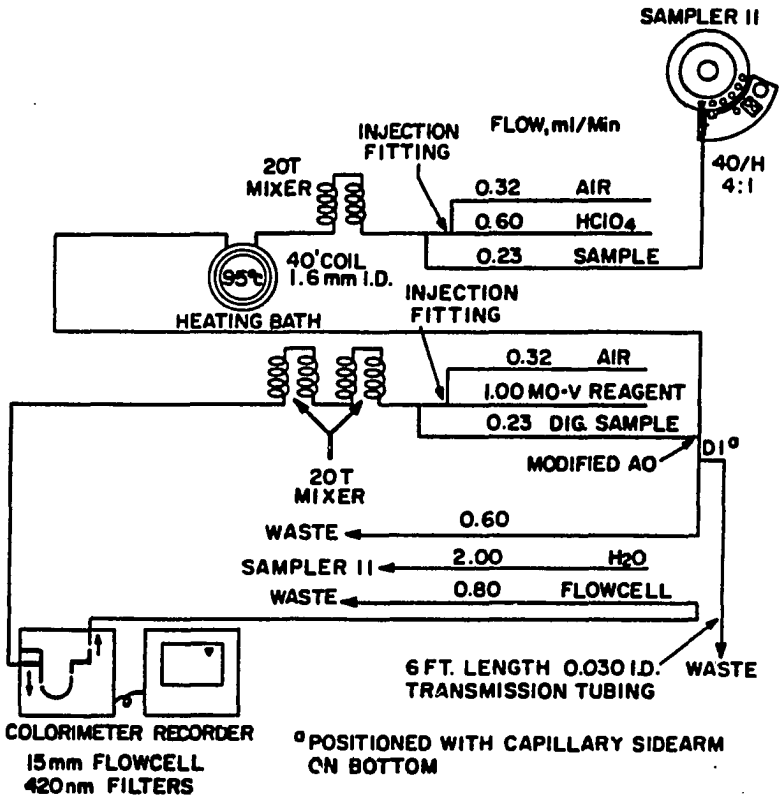
Excreta (.5 gram), digesta (.5 gram), and feed (1 gram) samples were weighed into porcelain crucibles and ashed overnight at 600°C in a muffle furnace. After ashing, the crucibles were cooled and 15 ml of 3N HCl were added to each crucible. The crucibles were heated on a hot plate to boiling for 30 to 60 seconds. The samples were transferred to 50 ml volumetric flasks and diluted to volume.

Five hundred milligrams of dried egg were weighed into porcelain crucibles and ashed for 24 hours at 600°C in a muffle furnace. After ashing, crucibles were cooled and 10 ml 3N HCl were added to each crucible. The crucibles were heated on a hot plate to boiling for 30 to 60 seconds. Samples were then transferred to 25 ml volumetric flasks and diluted to volume.

One hundred milligrams of bone ash were weighed into glass beakers. Fifteen mls of 3N HCl were added to each beaker and the beakers were heated on a hot plate to boiling for 30 to 60 seconds. Samples were transferred to a 50 ml volumetric flask and diluted to volume.

Aliquots of the diluted tissue, digesta, excreta, feed, egg and bone

preparations were analyzed for phosphorus by a Technicon Auto-Analyzer (Technicon Instruments Corp., Tarrytown, N.Y.). Phosphorus was reacted with a molybdovanadate reagent and measured at 424 nm in a flowcell within a range of 0.04 to 0.35 mg P/ml. The molybdovanadate reagent was prepared by the following steps: dissolve 8.25 g NH_4 molybdate in 400 ml hot H_2O, cool; dissolve 0.6 g NH_4 metavanadate in 250 ml hot H_{20}, cool; add 60 ml of 70% perchloric acid to the metavanadate solution and mix; gradually combine molybdate and metavanadate solutions with stirring; dilute to 2 liters. Phosphorus standards in the range of 0.04 to .35 mg P/ml were prepared from a Fisher (Fisher Scientific Co., FairLawn, N.J.) phosphate stock solution (1mg/ml). A linear standard curve was prepared with standard P concentrations plotted on the x axis and recorder peak height plotted on the y axis. Concentrations of phosphorus in samples of test materials were calculated by using the standard curve. A flow diagram of the auto-analyzer automated analysis for phosphorus is given below (Association of Official Analytical Chemists Methods, 1980).


SAMPLER II
INJECTION FITTING
FLOW, ml/Min
20T MIXER
0.32 AIR
0.60 HClO4
0.23 SAMPLE
40/H 4:1
95°C
40' COIL 1.6 mm I.D.
HEATING BATH
INJECTION FITTING
0.32 AIR
1.00 MO-V REAGENT
0.23 DIG. SAMPLE
MODIFIED AO
D1[a]
20T MIXER
WASTE
0.60
SAMPLER II
2.00 H2O
WASTE
0.80 FLOWCELL
6 FT. LENGTH 0.030 I.D. TRANSMISSION TUBING
WASTE
COLORIMETER RECORDER
15mm FLOWCELL
420nm FILTERS
[a] POSITIONED WITH CAPILLARY SIDEARM ON BOTTOM

APPENDIX C: PREPARATION OF EXCRETA AND FEED SAMPLES FOR PHYTATE AND PHYTATE PHOSPHORUS ANALYSIS

These procedures are modified from methods reported by Tangendjaja et al. (1980); Knuckles et al. (1982); and Common (1940). One gram of prepared dry excreta or 3 grams of prepared feed sample were weighed into a long test-tube. Twenty-five mls of 3% trichloroacetic acid were added to each test-tube. The contents of each tube were mixed on a mechanical wrist action shaker (Burrell Corp., Pittsburgh, PA.) for 2 hours. After mixing, samples were centrifuged at 2,000 RPM for 20 minutes. The supernatant was filtered through a 0.8 um Metricel (Gelman Sciences, Inc., Ann Arbor, MI.) membrane filter into a clean test-tube. An aliquot of the filtered solution was transferred to a 1 dram vial and stored for phytate determination by HPLC. The remaining supernatant was measured by pipette and transferred to a clean test-tube. One drop of phenolphthalein was added to each tube to function as a pH indicator. Drops of 25% NaOH were added to the solution while vortexing until the solution was slightly pink (basic). The solution was reacidified with a few drops of 0.5N HCl to a pale yellow color. Fifteen mls of a $FeCl_3$ solution (1 g $FeCl_3$ + 83 ml concentrated HCl diluted to 1 liter with H_2O) were added to each sample. The tubes were heated in a boiling water bath for 20 minutes. After cooling, the test-tubes were centrifuged for 20 minutes at 2,000 RPM. The supernatant was drained and 20 ml of 0.5N HCl was added to the precipitate and the tubes were centrifuged again for 20 minutes at 2,000 RPM. This last step was repeated twice to thoroughly wash the precipitate with 0.5N HCl.

The final supernatant was drained off and the precipitate was transferred to a 40 ml beaker by four serial additions of 5 ml concentrated nitric acid. Three glass boiling beads were also added to each beaker. The solution was then digested on a hot plate until only 10 ml of solution remained. The beakers were cooled on ice. After cooling, 4 ml concentrated sulfuric acid and 6 ml of concentrated perchloric acid were added to each beaker. The samples were again digested until the solution was clear and white fumes had evolved for 30 minutes (approximately 10 ml of solution remaining). Samples were cooled and transferred to a 25 ml volumetric flask and diluted to volume. The diluted samples were analyzed for phosphorus content by the Technicon Auto-Analyzer as described in Appendix B.

APPENDIX D: ANALYSIS OF PHYTATE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Excreta and feed samples were prepared for phytate analysis as described in Appendix C. Phytate was detected and quantified by high-performance liquid chromatography based on modified procedures from Tangendjaja et al. (1980) and Knuckles et al. (1982). A liquid chromatograph (Shimadzu model LC-4A, Kyoto, Japan) and an automated injector (Shimadzu model SIL-2A, Kyoto, Japan) were used to process the samples through a Versapack C18 column (300 MM x 4.1 MM) (Alltech Associates, Deerfield, IL.). Detection of phytate was done by using a differential refractive index detector (Waters Associates, Inc., Milford, MA.) and a chromatopac integrator (Shimadzu Model C-R3A, Kyoto, Japan).

Dipotassium phosphate (0.25 M and pH 6.0) was used for the mobile phase, followed by a methanol flush. Flow rate was 1.80 ml/minute and average retention time for phytate was 1.6 minutes. The time program used for the chromatograph and integrator was as follows:

Number	Time	Function	Value
00	0.00	Flow	1.80
01	0.00	P Max	200
02	0.00	Oven T	30
03	0.00	P Min	0
04	0.00	B Conc.	0.0
05	1.99	B Conc.	0.0
06	2.00	B Conc.	100.0
07	2.99	B Conc.	100.0
08	3.00	B Conc.	0.0
09	20.00	Stop	1.0

Standards were prepared from phytic acid (Inositol Hexaphosphoric Acid, Sigma Chemicals, St. Louis, MO.) in a linear range of .07 to .211% solutions. A linear standard curve was established with standard phytate

concentration on the x axis and peak area on the y axis. Phytate concentrations of excreta and feed samples were calculated from their peak area measurements as compared with peak areas of standard phytic acid solutions.

APPENDIX E: SPECTROPHOTOMETRIC PROCEDURE FOR THE DETERMINATION OF CHROMIC OXIDE

This procedure was modified from one reported by Fenton and Fenton (1979) for chromic oxide analysis in animal feed and feces. Excreta, digesta and feed were prepared for chromic oxide determination as described in Appendix A. One gram of excreta or digesta, or 2 grams of feed were weighed into a 30 ml glass beaker. Samples were ashed overnight in a muffle furnace at 450°C. After the samples cooled, 15 ml of a 150:150:200 mixture of distilled water: concentrated sulfuric acid: 70% perchloric acid, were pipetted into each beaker. Samples were heated on a hot plate until a yellowish or reddish color occurred for 10 to 15 minutes. Samples were removed from the hot plate and cooled, and then transferred to 100 ml volumetric flasks and diluted to volume. Ten ml of the diluted samples were transferred to disposable polystyrene culture tubes with polyethylene caps (Becton Dickenson Labware, Oxnard, CA.) and centrifuged for 5 minutes at 2,400 RPM.

The optical density was measured on a micro-sample spectrophotometer (Gilford 300N, Gilford Instruments Lab, Oberlin, OH.) at 440 nm. A standard curve was prepared by weighing 5 to 60 gram portions of pure Cr_2O_3 (Fisher Chromic Oxide Sesqui, Fisher Scientific Co., Fairlawn, N.J.) following the same procedure as previously described. A linear standard curve was plotted with chromium concentration on the x axis and optical density of the standards on the y axis. Chromic oxide concentrations of excreta, digesta and feed samples were calculated from this curve by using the optical density of the samples.

APPENDIX F: SCINTILLATION COUNTING OF ^{33}P IN EXCRETA, DIGESTA, TISSUES, EGG, BONE AND PLASMA

Samples were prepared for ^{33}P scintillation counting as described in Appendix B (preparation for phosphorus determination). One ml of each diluted sample preparation or 1 ml plasma was transferred to a 20 ml disposable glass scintillation vial (Kimble, Toledo, OH.) and 10 ml Beckman Ready-Solve EP (Beckman Instruments Inc., Fullerton, CA) liquid scintillation cocktail was added to each vial and mixed vigorously. Samples were counted for ^{33}P activity on a Packard Tri-Carb 300 liquid scintillation system (Packard Instrument Co., Downers Grove, Ill.). Windows were set in the region of 0 to 250 A and samples were counted for 20 minutes. Quench correction was done by a channels ratio method. Counting efficiency was greater than 92%. ^{33}P activity was expressed as DPM/tissue weight or DPM/mg P in tissue.

APPENDIX G: MEAN SQUARE TABLES

Table G1. Experiment one mean squares for egg production, egg weight, shell thickness, and feed intake

Source	d.f.	Mean Squares			
		Egg Production %	Egg Weight g	Shell Thickness mm	Feed Intake g/day
Calcium (Ca)	2	.2834	.18	.000140	6.32
Phosphorus (P)	2	.0795	.92	.000083	11.68
Ca x P	4	.0531	3.42	.0000103	8.24
Strain (S)	1	.1763	16.68[1]	.000790[2]	196.0[3]
S x Ca	2	.0178	1.52	.000004	1.65
S x P	2	.1163	.29	.000690[4]	6.74
S x Ca x P	4	.5141[5]	4.99	.000361	15.09
Error	27	.1447	2.71	.000232	23.69

[1] $p<.05$.
[2] $p<.08$.
[3] $p<.01$.
[4] $p<.07$.
[5] $p<.02$.

Table G2. Experiment one mean squares for P intake, Ca intake, femur ash, serum inorganic P and serum Ca

Source	d.f.	Mean Squares				
		P Intake mg/day	Ca Intake g/day	Femur Ash %	Serum P mg/dl	Serum Ca mg/dl
Calcium (Ca)	2	.21	.39868[1]	.168	.80	10.88
Phosphorus (P)	2	118.82[1]	.00109	.392[2]	13.70[1]	8.12
Ca x P	4	.23	.00080	.080	.86	59.52
Strain (S)	1	6.80[3]	.02337[3]	.394[3]	15.14[4]	212.09[5]
S x Ca	2	.07	.00003	.056	1.69	19.24
S x P	2	.38	.00085	.007	.65	71.83
S x Ca x P	4	.53	.00198	.056	.24	10.47
Error	27	.85	.00269	.114	.90	34.05

[1]p<.0001.
[2]p<.05.
[3]p<.01.
[4]p<.0003.
[5]p<.02.

Table G3: Experiment one mean squares for P retention at 34, 42, 50, 62, and 72 weeks of age

Source	d.f.	Mean Squares Age 34	42	50 mg/day	62	72
Calcium (Ca)	2	50610	12276	85341[1]	31052[2]	14950[2]
Phosphorus (P)	2	65824	24692[3]	32139	799	30330[4]
Ca x P	4	96781	35524[4]	34471	44288[4]	32707[5]
Strain (S)	1	89164[6]	7367	1606	608	12289
S x Ca	2	66056	4811	2844	1790	4389
S x P	2	40803	15093	14928	8865	8526
S x Ca x P	4	63014	28421	7487	8462	9590
Error	27	29223	8533	19550	9477	4638

[1] $p<.02$.
[2] $p<.06$.
[3] $p<.07$.
[4] $p<.01$.
[5] $p<.001$.
[6] $p<.08$.

Table G4. Experiment two mean squares for % phytate P retention at 34, 50, and 72 weeks of age

Source	d.f.	Mean Squares Age 34	50 %	72
Calcium (Ca)	2	530.24[1]	47.65	500.23
Phosphorus (P)	2	255.99	876.90[2]	813.91[1]
Ca x P	4	185.58	835.76[3]	929.12[4]
Strain (S)	1	4.17	599.49	459.33
S x Ca	2	108.55	492.14	33.57
S x P	2	210.72	344.45	139.98
S x Ca x P	4	152.31	545.98	290.26
Error	27	176.41	274.19	182.40

[1]$p<.07$.
[2]$p<.06$.
[3]$p<.08$.
[4]$p<.03$.

Table G5. Experiment two mean squares of % P associated with excreted phytate at 34, 50, and 72 weeks of age

Source	d.f.	Mean Squares Age 34	50 %	72
Calcium (Ca)	2	136.71	489.25[1]	48.53
Phosphorus (P)	2	73.13	6.67	1148.56[2]
Ca x P	4	31.57	194.62	349.86[3]
Strain (S)	1	304.50[4]	.003	11.36
S x Ca	2	226.39	19.18	2.32
S x P	2	114.17	16.33	5.01
S x Ca x P	4	44.76	29.81	291.17[4]
Error	27	91.82	110.15	56.91

[1] $p<.02$.
[2] $p<.0001$.
[3] $p<.04$.
[4] $p<.08$.